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(54) Cloned genes encoding IG-CD4 fusion proteins and the use thereof.

(57) Fusion proteins of immunoglobulins of the IgM, IgG1 or IgG3 class, wherein the variable region of the light or heavy chain has been replaced with CD4 or fragments thereof capable of binding to gp120 or immunoglobulin-like molecules comprising such fusion proteins together with an immunoglobulin light or heavy chain can be administered to an animal suffering from HIV or SIV infection. They also are useful in assays for HIV or SIV comprising contacting a sample suspected of containing HIV or SIV gp120 with the immunoglobulin-like molecule or fusion protein, and detecting whether a complex is formed.

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CLONED GENES ENCODING IG-CD4 FUSION PROTEINS AND THE USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. Application Serial No. 07/147,351 filed January 22, 1988.

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FIELD OF THE INVENTION

The invention is in the field of recombinant genetics.

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BACKGROUND OF THE INVENTION

The human and simian immunodeficiency viruses HIV and SIV are the causative agents of Acquired
15 Immune Deficiency Syndrome (AIDS) and Simian Immunodeficiency Syndrome (SIDS), respectively. See Curren, J. et al., Science 329:1359-1357 (1985); Weiss, R. et al., Nature 324:572-575 (1986). The HIV virus contains an envelope glycoprotein, gp120 which binds to the CD4 protein present on the surface of helper T lymphocytes, macrophages and other cells. Dalgleish et al. Nature, 312:763 (1984). After the gp120 binds to CD4, virus entry is facilitated by an envelope-mediated fusion of the viral target cell membranes.
20 During the course of infection, the host organism develops antibodies against viral proteins, including the major envelope glycoproteins gp120 and gp41. Despite this humoral immunity, the disease progresses, resulting in a lethal immunosuppression characterized by multiple opportunistic infections, parasitemia, dementia and death. The failure of host anti-viral antibodies to arrest the progression of the disease represents one of the most vexing and alarming aspects of the infection, and augurs poorly for vaccination
25 efforts based upon conventional approaches.

Two factors may play a role in the inefficacy of the humoral response to immunodeficiency viruses. First, like other RNA viruses (and like retroviruses in particular), the immunodeficiency viruses show a high mutation rate which allows antigenic variation to progress at a high rate in response to host immune surveillance. Second, the envelope glycoproteins themselves are heavily glycosylated molecules presenting
30 few epitopes suitable for high affinity antibody binding. The poorly antigenic, "moving" target which the viral envelope presents, allows the host little opportunity for restricting viral infection by specific antibody production.

Cells infected by the HIV virus express the gp120 glycoprotein on their surface. Gp120 mediates fusion events among CD4⁺ cells via a reaction similar to that by which the virus enters the uninfected cell, leading
35 to the formation of short-lived multinucleated giant cells. Syncytium formation is dependent on a direct interaction of the gp120 envelope glycoprotein with the CD4 protein. Dalgleish et al., supra, Klatzmann, D. et al., Nature 312:763 (1984); McDougal, J.S. et al. Science, 231:382 (1986); Sodroski, J. et al., Nature, 322:470 (1986); Lifson, J.D. et al., Nature, 323:725 (1986); Sodroski, J. et al., Nature, 321:412 (1986).

The CD4 protein consists of a 370 amino acid extracellular region containing four immunoglobulin-like
40 domains, a membrane spanning domain, and a charged intracellular region of 40 amino acid residues. Maddon, P. et al., Cell 42:93 (1985); Clark, S. et al., Proc. Natl. Acad. Sci. (USA) 84:1649 (1987).

Evidence that CD4-gp120 binding is responsible for viral infection of cells bearing the CD4 antigen includes the finding that a specific complex is formed between gp120 and CD4. McDougal et al., supra. Other workers have shown that cell lines, which were non-infective for HIV, were converted to infectable cell
45 lines following transfection and expression of the human CD4 cDNA gene. Maddon et al., Cell 47:333-348 (1986).

In contrast to the majority of antibody-envelope interactions, the receptor-envelope interaction is characterized by a high affinity ($K_a \approx 10^8$ l/mole) immutable association. Moreover, the affinity of the virus for CD4 is at least 3 orders of magnitude higher than the affinity of CD4 for its putative endogenous ligand, the
50 MHC class II antigens. Indeed, to date, a specific physical association between monomeric CD4 and class II antigens has not been demonstrated.

In response to bacterial or other particle infection, the host organism usually produces serum antibodies that bind to specific proteins or carbohydrates on the bacterial or particle surface, coating the bacteria. This antibody coat on the bacterium or other particle stimulates cytolysis by Fc-receptor-bearing lymphoid cells by antibody-dependent cellular toxicity (ADCC). Other serum proteins, collectively called complement (C),

bind to antibody-coated targets, and also can coat foreign particles nonspecifically. They cause cell death by lysis, or stimulate ingestion by binding to specific receptors on the macrophage called complement receptors. See Darnell J. et al., in Molecular Cell Biology, Scientific American Books, pp. 641 and 1087 (1986).

5   The most effective complement activating classes of human Ig are IgM and IgG1. The complement system consists of 14 proteins that, acting in order, cause lysis of cells. Nearly all of the C proteins exist in normal serum as inactive precursors. When activated, some become highly specific proteolytic enzymes whose substrate is the next protein in a sequential chain reaction.

The entire C sequence can be triggered by either of two initiation pathways. In one (the classic
10   pathway), Ab-Ag complexes bind and activate C1, C4 and C2 to form a C3-splitting enzyme. In the second pathway, polysaccharides commonly on the surface of many bacteria and fungi bind with trace amounts of a C3 fragment and then with two other proteins (factor B and properdin) to form another C3-splitting enzyme. Once C3 is split by either pathway, the way is open for the remaining sequence of steps which lead to cell lysis. See Davis, B.D., et al., in Microbiology, 3rd ed., Harper and Row, Philadelphia, PA, pp.
15   452-466 (1980).

A number of workers have disclosed methods for preparing hybrid proteins. For example, Murphy, United States Patent 4,675,382 (1987), discloses the use of recombinant DNA techniques to make hybrid protein molecules by forming the desired fused gene coding for a hybrid protein of diptheria toxin and a polypeptide ligand such as a hormone, followed by expression of the fused gene.

20   Many workers have prepared monoclonal antibodies (Mabs) by recombinant DNA techniques. Monoclonal antibodies are highly specific well-characterized molecules in both primary and tertiary structure. They have been widely used for in vitro immunochemical characterization and quantitation of antigens. Genes for heavy and light chains have been introduced into appropriate hosts and expressed, followed by reaggregation of the individual chains into functional antibody molecules (see, for example, Munro, Nature
25   312:597 (1984); Morrison, S.L., Science 229:1202 (1985); Oi et al., Biotechniques 4:214 (1986); Wood et al., Nature 314:446-449 (1985)). Light- and heavy-chain variable regions have been cloned and expressed in foreign hosts wherein they maintained their binding ability (Moore et al., European Patent Application 0088994 (published September 21, 1983)).

Chimeric or hybrid antibodies have also been prepared by recombinant DNA techniques. Oi and
30   Morrison, Biotechniques 4:214 (1986) describe a strategy for producing such chimeric antibodies which include a chimeric human IgG anti-leu3 antibody.

Gascoigne, N.R.J., et al., Proc. Natl. Acad. Sci. (USA) 84:2936-2940 (1987) disclose the preparation of a chimeric gene construct containing a T-cell receptor a-chain variable (V) domain and the constant (C) region coding sequence of an immunoglobulin γ2a molecule. Cells transfected with the chimeric gene synthesize a
35   protein product that expresses immunoglobulin and T-cell receptor antigenic determinants as well as protein A binding sites. This protein associates with a normal λ chain to form an apparently normal tetrameric (H_2L_2, where H=heavy and L=light) immunoglobulin molecule that is secreted.

Sharon, J., et al., Nature 309:54 (1984), disclose construction of a chimeric gene encoding the variable (V) region of a mouse heavy chain specific for the hapten azophenylarsonate and the constant (C) region of
40   a mouse kappa light chain (V_HC_K). This gene was introduced into a mouse myeloma cell line. The chimeric gene was expressed to give a protein which associated with light chains secreted from the myeloma cell line to give an antibody molecule specific for azophenylarsonate.

Morrison, Science 229:1202 (1985), discloses that variable light-or variable heavy-chain regions can be attached to a non-Ig sequence to create fusion proteins. This article states that the potential uses for the
45   fusion proteins are three: (1) to attach antibody specifically to enzymes for use in assays; (2) to isolate non-Ig proteins by antigen columns; and (3) to specifically deliver toxic agents.

Recent techniques for the stable introduction of immunoglobulin genes into myeloma cells (Banerji, J., et al., Cell 33:729-740 (1983); Potter, H., et al., Proc. Natl. Acad. Sci. (USA) 81:7161-7165 (1984)), coupled with detailed structural information, have permitted the use of in vitro DNA methods such as mutagenesis,
50   to generate recombinant antibodies possessing novel properties.

PCT Application W087/02671 discloses methods for producing genetically engineered antibodies of desired variable region specificity and constant region properties through gene cloning and expression of light and heavy chains. The mRNA from cloned hybridoma B cell lines which produce monoclonal antibodies of desired specificity is isolated for cDNA cloning. The generation of light and heavy chain
55   coding sequences is accomplished by excising the cloned variable regions and ligating them to light or heavy chain module vectors. This gives cDNA sequences which code for immunoglobulin chains. The lack of introns allows these cDNA sequences to be expressed in prokaryotic hosts, such as bacteria, or in lower eukaryotic hosts, such as yeast.

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The generation of chimeric antibodies in which the antigen-binding portion of the immunoglobulin is fused to other moieties has been demonstrated. Examples of non-immunoglobulin genes fused to antibodies include Stanphylococcus aureus nuclease, the mouse oncogene c-myc, and the Klenow fragment of E. coli DNA polymerase I (Neuberger, M.S., et al., Nature 312:604-612 (1984); Neuberger, M.S., Trends in Biochemical Science, 347-349 (1985)). European Patent Application 120,694 discloses the genetic engineering of the variable and constant regions of an immunoglobulin molecule that is expressed in E. coli host cells. It is further disclosed that the immunoglobulin molecule may be synthesized by a host cell with another peptide moiety attached to one of the constant domains. Such peptide moieties are described as either cytotoxic or enzymatic. The application and the examples describe the use of a lambda-like chain derived from a monoclonal antibody which binds to 4-hydroxy-3-nitrophenyl (NP) haptens.

European Patent Application 125,023 relates to the use of recombinant DNA techniques to produce immunoglobulin molecules that are chimeric or otherwise modified. One of the uses described for these immunoglobulin molecules is for whole-body diagnosis and treatment by injection of the antibodies directed to specific target tissues. The presence of the disease can be determined by attaching a suitable label to the antibodies, or the diseased tissue can be attacked by carrying a suitable drug with the antibodies. The application describes antibodies engineered to aid the specific delivery of an agent as "altered antibodies."

PCT Application WO83/101533 describes chimeric antibodies wherein the variable region of an immunoglobulin molecule is linked to a portion of a second protein which may comprise the active portion of an enzyme.

Boulianne et al., Nature-312:643 (1984) constructed an immunoglobulin gene in which the DNA segments that encode mouse variable regions specific for the hapten trinitrophenol (TNP) are joined to segments that encode human mu and kappa regions. These chimeric genes were expressed to give functional TNP-binding chimeric IgM.

Morrison et al., P.N.A.S. (USA) 81:6851 (1984), disclose a chimeric molecule utilizing the heavy-chain variable region exons of an anti-phosphoryl choline myeloma protein G, which were joined to the exons of either human kappa light-chain gene. The genes were transfected into mouse myeloma cell lines, generating transformed cells that produced chimeric mouse-human IgG with antigen-binding function.

Despite the progress that has been achieved on determining the mechanism of HIV infection, a need continues to exist for methods of treating HIV viral infections.

SUMMARY OF THE INVENTION

The invention relates to a gene comprising a DNA sequence which encodes a fusion protein comprising 1) CD4, or a fragment thereof which binds to HIV gp120, and 2) an immunoglobulin light or heavy chain; wherein said CD4 or HIV gp120-binding fragment thereof replaces the variable region of the light or heavy immunoglobulin chain.

The invention also relates to vectors containing the gene of the invention and hosts transformed with the vectors.

The invention also relates to a method of producing a fusion protein comprising CD4, or fragment thereof which binds to HIV gp120, and an immunoglobulin light or heavy chain, wherein the variable region of the immunoglobulin light or heavy chain has been substituted with CD4, or HIV gp120-binding fragment thereof, which comprises:

cultivating in a nutrient medium under protein producing conditions, a host strain transformed with the vector containing the gene of the invention, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and
recovering the fusion protein so produced.

The invention also relates to a fusion protein comprising CD4, or fragment thereof which is capable of binding to HIV gp120, fused at the C-terminus to a second protein which comprises an immunoglobulin light or heavy chain, wherein the variable region of said light or heavy chain is substituted with CD4 or a HIV gp120 binding fragment thereof.

The invention also relates to an immunoglobulin-like molecule comprising the fusion protein of the invention together with an immunoglobulin light or heavy-chain, wherein said immunoglobulin like molecule binds HIV gp120.

The IgG1 fusion proteins and immunoglobulin-like molecules may be useful for both complement-mediated and cell-mediated (ADCC) immunity, while the IgM fusion proteins are useful principally through complement-mediated immunity.

The invention also relates to a complex between the fusion proteins and immunoglobulin-like molecule of the invention and HIV gp120.

The invention also relates to a method for treating HIV or SIV infections comprising administering the fusion protein or immunoglobulin-like molecule of the invention to an animal.

The invention further relates to a method for detecting HIV gp120 in a sample comprising contacting a sample suspected of containing HIV or gp120 with the fusion protein or immunoglobulin-like molecule of the invention, and detecting whether a complex has formed.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention is directed to a protein gene which comprises
1) a DNA sequence which codes for CD4, or fragment thereof which binds to HIV gp120, fused to
2) a DNA sequence which encodes an immunoglobulin heavy chain.

Preferably, the antibody has effector function.
The invention is also directed to a protein gene which comprises
1) a DNA sequence which codes for CD4, or fragment thereof which binds to HIV gp120, fused to
2) a DNA sequence which encodes an immunoglobulin light chain; wherein said sequence which codes for CD4, or HIV gp120-binding fragment thereof, replaces the variable region of the light immunoglobulin chain.

The invention is also directed to the expression of these novel fusion proteins in transformed hosts and the use thereof to treat and diagnose HIV infections. In particular, the invention relates to expressing said genes in mammalian hosts which express complementary light or heavy chain immunoglobulins to give immunoglobulin-like molecules which have antibody effector function and also bind to HIV or SIV gp120.

The term "antibody effector function" as used herein denotes the ability to fix complement or to activate ADCC.

The fusion proteins and immunoglobulin-like molecules may be administered to an animal for the purpose of treating HIV or SIV infections. By the terms "HIV infections" is intended the condition of having AIDS, AIDS related complex (ARC) or where an animal harbors the AIDS virus, but does not exhibit the clinical symptoms of AIDS or ARC. By the terms "SIV infections" is intended the condition of being infected with simian immunodeficiency virus.

By the term "animal" is intended all animals which may derive benefit from the administration of the fusion proteins and immunoglobulin-like molecules of the invention. Foremost among such animals are humans, however, the invention is not intended to be so limited.

By the term "fusion protein" is intended a fused protein comprising CD4, or fragment thereof which is capable of binding to gp120, linked at its C-terminus to an immunoglobulin chain wherein a portion of the N-terminus of the immunoglobulin is replaced with CD4. In general, that portion of immunoglobulin which is deleted is the variable region. The fusion proteins of the invention may also comprise immunoglobulins where more than just the variable region has been deleted and replaced with CD4 or HIV gp120 binding fragment thereof. For example, the V_H and CH1 regions of an immunoglobulin chain may be deleted. Preferably, any amount of the N-terminus of the immunoglobulin heavy chain can be deleted as long as the remaining fragment has antibody effector function. The minimum sequence required for binding complement encompasses domains CH2 and CH3. Joining of Fc portions by the hinge region is advantageous for increasing the efficiency of complement binding.

The CD4 portion of the fusion protein may comprise the complete CD4 sequence, the 370 amino acid extracellular region and the membrane spanning domain, or the extracellular region. The fusion protein may comprise fragments of the extracellular region obtained by cutting the DNA sequence which encodes CD4 at the BspM1 site at position 514 or the PvuII site at position 629 (see Table 1) to give nucleotide sequences which encode CD4 fragments which retain binding to gp120. In general, any fragment of CD4 may be used as long as it retains binding to gp120.

Where the fusion protein comprises an immunoglobulin light chain, it is necessary that no more of the Ig chain be deleted than is necessary to form a stable complex with a heavy chain Ig. In particu lar, the cysteine residues necessary for disulfide bond formation must be preserved on both the heavy and light chain moieties.

When expressed in a host, e.g., a mammalian cell, the fusion protein may associate with other light or

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heavy Ig chains secreted by the cell to give a functioning immunoglobulin-like molecule which is capable of binding to gp120. The gp120 may be in solution, expressed on the surface of infected cells, or may be present on the surface of the HIV virus itself. Alternatively, the fusion protein may be expressed in a mammalian cell which does not secrete other light or heavy Ig chains. When expressed under these

5 conditions, the fusion protein may form a homodimer.

Genomic or CDNA sequences may be used in the practice of the invention. Genomic sequences are expressed efficiently in myeloma cells, since they contain native promoter structures.

The constant regions of the antibody cloned and used in the chimeric immunoglobulin-like molecule may be derived from any mammalian source. The constant regions may be complement binding or ADCC

10 active. However, preliminary work (see Examples) indicates that the fusion proteins of the invention may mediate HIV or SIV infected cell death by an ADCC or complement-independent mechanism. The constant regions may be derived from any appropriate isotype, including IgG1, IgG3, or IgM.

The joining of various DNA fragments, is performed in accordance with conventional techniques, employing blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide

15 appropriate termini, filling in of cohesive ends as appropriate, alkali and phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. The genetic construct may optionally encode a leader sequence to allow efficient expression of the fusion protein. For example, the leader sequence utilized by Maddon et al., Cell 42:93-104 (1985) for the expression of CD4 may be used.

For cDNA, the cDNA may be cloned and the resulting clone screened, for example, by use of a

20 complementary probe or by assay for expressed CD4 using an antibody as disclosed by Daigleish et al., Nature 312:763-766 (1984); Klatzmann et al., Immunol. Today 7:291-297 (1986); McDougal et al., J. Immunol. 135:3151-3162 (1985); and McDougal, J. et al., J. Immunol. 137:2937-2944 (1986).

To express the fusion hybrid protein, transcriptional and translational signals recognized by an appropriate host element are necessary. Eukaryotic hosts which may be used include mammalian cells

25 capable of culture in vitro, particularly leukocytes, more particularly myeloma cells or other transformed or oncogenic lymphocytes, e.g., EBV-transformed cells. Alternatively, non-mammalian cells may be employed, such as bacteria, fungi, e.g., yeast, filamentous fungi, or the like.

Preferred hosts for fusion protein production are mammalian cells, grown in vitro in tissue culture or in vivo in animals. Mammalian cells provide post translational modification to immunoglobulin protein mol-

30 ecules which provide for correct folding and glycosylation of appropriate sites. Mammalian cells which may be useful as hosts include cells of fibroblast origins such as VERO or CHO-K1 or cells of lymphoid origin, such as the hybridoma SP2/0-AG14 or the myeloma P3x63Sgh, and their derivatives. For the purpose of preparing an immunoglobulin-like molecule, a plasmid containing a gene which encodes a heavy chain immunoglobulin, wherein the variable region has been replaced with CD4 or fragment thereof which binds to

35 gp120, may be introduced, for example, into J558L myeloma cells, a mouse plasmacytoma expressing the lambda-1 light chain but which does not express a heavy chain (see Oi et al., P.N.A.S. (USA) 80:825-829 (1983)). Other preferred hosts include COS cells, BHK cells and hepatoma cells.

The constructs may be joined together to form a single DNA segment or may be maintained as separate segments, by themselves or in conjunction with vectors.

40 Where the fusion protein is not glycosylated, any host may be used to express the protein which is compatible with replicon and control sequences in the expression plasmid. In general, vectors containing replicon and control sequences are derived from species compatible with a host cell are used in connection with the host. The vector ordinarily carries a replicon site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. The expression of the fusion protein can also be placed

45 under control with other regulatory sequences which may be homologous to the organism in its untransformed state. For example, lactose-dependent E. coli chromosomal DNA comprises a lactose or lac operon which mediates lactose utilization by elaborating the enzyme beta-galactosidase. The lac control elements may be obtained from bacterial phage lambda plac5, which is infective for E. coli. The lac promoter-operator system can be induced by IPTG.

50 Other promoters/operator systems or portions thereof can be employed as well. For example, colicin E1, galactose, alkaline phosphatase, tryptophan, xylose, tax, and the like can be used.

For mammalian hosts, several possible vector systems are available for expression. One class of vectors utilize DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses RSV, MMTV or MOMLV), or SV40 virus.

55 Cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototropy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals such as copper or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced

into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals. The cDNA expression vectors incorporating such elements includes those described by Okayama, H., Mol. Cel. Biol., 3:280 (1983) and others.

Once the vector or DNA sequence containing the constructs has been prepared for expression, the DNA constructs may be introduced to an appropriate host. Various techniques may be employed, such as protoplast fusion, calcium phosphate precipitation, electroporation or other conventional techniques. After the fusion, the cells are grown in media and screened for the appropriate activity. Expression of the gene(s) results in production of the fusion protein. This expressed fusion protein may then be subject to further assembly to form the immunoglobulin-like molecule.

The host cells for immunoglobulin production may be immortalized cells, primarily myeloma or lymphoma cells. These cells may be grown in appropriate nutrient medium in culture flasks or injected into a synergistic host, e.g., mouse or a rat, or immunodeficient host or host site, e.g., nude mouse or hamster pouch. In particular, the cells may be introduced into the abdominal cavity of an animal to allow production of ascites fluid which contains the immunoglobulin-like molecule. Alternatively, the cells may be injected subcutaneously and the chimeric antibody is harvested from the blood of the host. The cells may be used in the same manner as hybridoma cells. See Diamond et al., N. Eng. J. Med. 304:1344 (1981), and Kennatt, McKearn and Bechtol (Eds.), Monoclonal Antibodies: Hybridomas: – A New Dimension in Biologic Analysis, Plenum, 1980.

The fusion proteins and immunoglobulin-like molecules of the invention may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis or the like. For example, the IgG1 fusion proteins may be purified by passing a solution through a column which contains immobilized protein A or protein G which selectively binds the Fc portion of the fusion protein. See, for example, Reis, K.J., et al., J. Immunol. 132:3098-3102 (1984); PCT Application, Publication No. W087/00329. The chimeric antibody may the be eluted by treatment with a chaotropic salt or by elution with aqueous acetic acid (1 M).

Alternatively the fusion proteins may be purified on anti-CD4 antibody columns, or on anti-immunoglobulin antibody columns.

In one embodiment of the invention, cDNA sequences which encode CD4, or a fragment thereof which binds gp120, may be ligated into an expression plasmid which codes for an antibody wherein the variable region of the gene has been deleted. Methods for the preparation of genes which encode the heavy or light chain constant regions of immunoglobulins are taught, for example, by Robinson, R. et al., PCT Application, Publication No. W087-02671.

Preferred immunoglobulin-like molecules which contain CD4, or fragments thereof, contain the constant region of an IgM, IgG1 or IgG3 antibody which binds complement at the Fc region.

The fusion protein and immunoglobulin-like molecules of the invention may be used for the treatment of HIV viral infections. The fusion protein complexes to gp120 which is expressed on infected cells. Although the inventor is not bound by a particular theory, it appears that the Fc portion of the hybrid fusion protein may bind with complement, which mediates destruction of the cell. In this manner, infected cells are destroyed so that additional viral particle production is stopped.

For the purpose of treating HIV infections, the fusion protein or immunoglobulin-like molecule of the invention may additionally contain a radiolabel or therapeutic agent which enhances destruction of the HIV particle or HIV-infected cell.

Examples of radioisotopes which can be bound to the fusion protein or immunoglobulin-like molecule of the invention for use in HIV-therapy are ^{125}I, ^{131}I, ^{90}Y, ^{67}Cu, ^{217}Bi, ^{211}At, ^{212}Pb, ^{47}Sc, and ^{109}Pd. Optionally, a label such as boron can be used which emits α and β particles upon bombardment with neutron radiation.

For in vivo diagnosis radionucleotides may be bound to the fusion protein or immunoglobulin-like molecule of the invention either directly or by using an intermediary functional group. An intermediary group which is often used to bind radioisotopes, which exist as metallic cations, to antibodies is diethylenetriaminepentaacetic acid (DTPA). Typical examples of metallic cations which are bound in this manner are ^{99m}Tc ^{123}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, and ^{68}Ga.

Moreover, the fusion protein and immunoglobulin-like molecule of the invention may be tagged with an NMR imaging agent which include paramagnetic atoms. The use of an NMR imaging agent allows the in vivo diagnosis of the presence of and the extent of HIV infection within a patient using NMR techniques. Elements which are particularly useful in this manner are ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

Therapeutic agents may include, for example, bacterial toxins such as diphtheria toxin, or ricin. Methods for producing fusion proteins comprising fragment A of diphtheria toxin are taught in U.S. Patent 4,675,382 (1987). Diphtheria toxin contains two polypeptide chains. The B chain binds the toxin to a receptor on a cell

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surface. The A chain actually enters the cytoplasm and inhibits protein synthesis by inactivating elongation factor 2, the factor that translocates ribosomes along mRNA concomitant with hydrolysis of ETP. See Darnell, J., et al., in Molecular Cell Biology, Scientific American Books, Inc., page 662 (1986). Alternatively, a fusion protein comprising ricin, a toxic lectin, may be prepared.

Introduction of the chimeric molecules by gene therapy may also be contemplated, for example, using retroviruses or other means to introduce the genetic material encoding the fusion proteins into suitable target tissues. In this embodiment, the target tissues having the cloned genes of the invention may then produce the fusion protein in vivo.

The dose ranges for the administration of the fusion protein or immunoglobulin-like molecule of the invention are those which are large enough to produce the desired effect whereby the symptoms of HIV or SIV infection are ameliorated. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counterindications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. Dosage can vary from .01 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 1.0 mg/kg, of the immunoglobulin-like molecule in one or more administrations daily, for one or several days. The immunoglobulin-like molecule can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like. See, generally, Remington's Pharmaceutical Science, 16th Ed., Mack Eds., 1980.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising the components of the invention, the medicament being used for therapy of HIV or SIV infection in animals.

The detection and quantitation of antigenic substances and biological samples frequently utilized immunoassay techniques. These techniques are based upon the formation of the complex between the antigenic substance, e.g., gp120, being assayed and an antibody or antibodies in which one or the other member of the complex may be detectably labeled. In the present invention, the immunoglobulin-like molecule or fusion protein may be labeled with any conventional label.

Thus, the hybrid fusion protein or immunoglobulin-like molecule of the invention can also be used in assay for HIV or SIV viral infection in a biological sample by contacting a sample, derived from an animal suspected of having an HIV or SIV infection, with the fusion protein or immunoglobulin-like molecule of the invention, and detecting whether a complex with gp120, either alone or on the surface of an HIV-infected cell, has formed.

For example, a biological sample may be treated with nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble protein. The support may then be washed with suitable buffers followed by treatment with the fusion protein which may be detectably labeled. The solid phase support may then be washed with the buffer a second time to remove unbound fusion protein and the label on the fusion protein detected.

In carrying out the assay of the present invention on a sample containing gp120, the process comprises:

a) contacting a sample suspected containing gp120 with a solid support to effect immobilization of gp120, or cell which expresses gp120 on its surface;

b) contacting said solid support with the detectably labeled immunoglobulin-like molecule or fusion protein of the invention;

c) incubating said detectably labeled immunoglobulin-like molecule with said support for a sufficient amount of time to allow the immunoglobulin-like molecule or fusion protein to bind to the immobilized gp120 or cell which expresses gp120 on its surface;

d) separating the solid phase support from the incubation mixture obtained in step c); and

e) detecting the bound immunoglobulin-like molecule or fusion protein and thereby detecting and quantifying gp120.

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Alternatively, labeled immunoglobulin-like molecule (or fusion protein) -gp120 complex in a sample may be separated from a reaction mixture by contacting the complex with an immobilized antibody or protein which is specific for an immunoglobulin or, e.g., protein A, protein G, anti-IgM or anti-IgG antibodies. Such anti-immunoglobulin antibodies may be monoclonal or polyclonal. The solid support may then be washed with suitable buffers to give an immobilized gp120-labeled immunoglobulin-like molecule antibody complex. The label on the fusion protein may then be detected to give a measure of endogenous gp120 and, thereby, the presence of HIV.

This aspect of the invention relates to a method for detecting HIV or SIV viral infection in a sample comprising

(a) contacting a sample suspected of containing gp120 with a fusion protein or immunoglobulin-like molecule comprising CD4, or fragment thereof which binds to gp120, and the Fc portion of an immunoglobulin chain,

(b) detecting whether a complex is formed.

The invention also relates to a method of detecting gp120 in a sample, further comprising

(c) contacting the mixture obtained in step (a) with an Fc binding molecule, such as an antibody, protein A, or protein G, which is immobilized on a solid phase support and is specific for the hybrid fusion protein, to give a gp120 fusion protein-immobilized antibody complex

(d) washing the solid phase support obtained in step (c) to remove unbound fusion protein,

(e) and detecting the label on the hybrid fusion protein.

Of course, the specific concentrations of detectably labeled immunoglobulin-like molecule (or fusion protein) and gp120, the temperature and time of incubation, as well as other assay conditions may be varied, depending on various factors including the concentration of gp120 in the sample, the nature of the sample, and the like. Those skilled in the art wild be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

One of the ways in which the immunoglobulin-like molecule or fusion protein of the present invention can be detectably labeled is by linking the same to an enzyme. This enzyme, in turn, when later exposed to its substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected as, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the immunoglobulin-like molecule or fusion protein of the present invention include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholine esterase.

The immunoglobulin-like molecule or fusion protein of the present invention may also be labeled with a radioactive isotope which can be determined by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention are: ^{3}H, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{58}Co, ^{59}Fe and ^{75}Se.

It is also possible to label the immunoglobulin-like molecule or fusion protein with a fluorescent compound. When the fluorescently labeled immunoglobulin-like molecule is exposed to light of the proper wave length, its presence can then be detected due to the fluorescence of the dye. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The immunoglobulin-like molecule or fusion protein of the invention can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the immunoglobulin-like molecule or fusion protein using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The immunoglobulin-like molecule or fusion protein of the present invention also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoglobulin-like molecule or fusion protein is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the immunoglobulin-like molecule or fusion protein of the present invention. Bioluminescence is a type of chemiluminescence found in biological

systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Detection of the immunoglobulin-like molecule or fusion protein may be accomplished by a scintillation counter, for example, if the detectable label is a radioactive gamma emitter, or by a fluorometer, for example, if the label is a fluorescent material. In the case of an enzyme label, the detection can be accomplished by colorimetric methods which employ a substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

The assay of the present invention is ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement therewith one or more container means such as vials, tubes and the like, each of said container means comprising the separate elements of the immunoassay. For example, there may be a container means containing a solid phase support, and further container means containing the detectably labeled immunoglobulin-like molecule or fusion protein in solution. Further container means may contain standard solutions comprising serial dilutions of analytes such as gp120 or fragments thereof to be detected. The standard solutions of these analytes may be used to prepare a standard curve with the concentration of gp120 plotted on the abscissa and the detection signal on the ordinate. The results obtained from a sample containing gp120 may be interpolated from such a plot to give the concentration of gp120.

The immunoglobulin-like molecule or fusion protein of the present invention can also be used as a stain for tissue sections. For example, a labeled immunoglobulin-like molecule comprising CD4 or fragment thereof which binds to gp120 may be contacted with a tissue section, e.g., a brain biopsy specimen. This section may then be washed and the label detected.

The following examples are illustrative, but not limiting the method and composition of the present invention. Other suitable modifications and adaptations which are obvious to this skill in the art are within the spirit and scope of this invention.

EXAMPLES

Example 1: Preparation of CD4-Ig cDNA Constructs

The extracellular portion of the CD4 molecule (See Madden, P.J., et al., Cell 42:93-104 (1985)) was fused at three locations in a human IgG1 heavy chain constant region gene by means of a synthetic splice donor linker molecule. To exploit the splice donor linker, a BamHI linker having the sequence CGCGGATCCGCG was first inserted at amino acid residue 395 of the CD4 precursor sequence (nucleotide residue 1295). A synthetic splice donor sequence

$$\text{GATCCCGAGGGTGAGTACTA}$$
$$\text{GGCTCCCACTCATGATTCGA}$$

bounded by BamHI and HindIII complementary ends was created and fused to the HindIII site in the intron preceding the CH1 domain, to the EspI site in the intron preceding the hinge domain, and to the BanI site preceding the CH2 domain of the IgG1 genomic sequence. Assembly of the chimeric genes by ligation at the BamHI site afforded molecules in which either the variable (V) region, the V+CH1 regions, or the V, CH1 and hinge regions were replaced by CD4. In the last case, the chimeric molecule is expected to form a monomer structure, while in the former, a dimeric molecule is expected.

On such genetic construct which contains the DNA sequence which encodes CD4 linked to human IgG1 at the Hind3 site upstream of the CH1 region (fusion protein CD4Hγ1) is depicted in Table 1. The plasmid containing this genetic construct (pCD4Hγ1) has been deposited in E. coli (MC1061/P3) at the American Type Culture Collection (ATCC) under the terms of the Budapest Treaty and given accession number 67611.

A second genetic construct which contains the DNA sequence which encodes CD4 linked to human IgG1 at the Esp site upstream of the hinge region (fusion protein CD4Eγ1) is depicted in Table 2. The

plasmid containing this genetic construct (pCD4Eγ1) has been deposited in E. coli (MC1061/P3) at the ATCC under the terms of the Budapest Treaty and given accession number 67610.

A third genetic construct which contains the DNA sequence which encodes CD4 linked to human IgM at the Mst2 site upstream of the CH1 region (fusion protein CD4Mμ) is depicted in Table 3. The plasmid

5 containing this genetic construct (PCD4Mμ) has been deposited in E. coli (MC1061/P3) at the ATCC under the terms of the Budapest Treaty and given accession number 67608.

A fourth genetic construct which contains the DNA sequence which encodes CD4 linked to human IgM at the Pst site upstream of the CH2 region (fusion protein CD4Pμ) is depicted in Table 4. The plasmid containing this genetic construct (PCD4Pμ) has been deposited in E. coli (MC1061/P3) at the ATCC under

10 the terms of the Budapest Treaty and given accession number 67609.

A fifth genetic construct which contains the DNA sequence which encodes CD4 linked to human IgG1 at the Ban1 site downstream from the hinge region (fusion protein CD4Bγ1) is depicted in Table 5.

Two similar constructs were prepared from the human IgM heavy chain constant region by fusion with the introns upstream of the μ CH1 and CH2 domains at an MStII site and a PStI site respectively. The

15 fusions were made by joining the PStI site of the CD4/IgG1 construct fused at the Esp site in IgG1 gene to the MStII and Pst sites in the IgM gene. In the first instance, this was performed by treatment of the Pst end with T4 DNA Polymerase and the MStII end with E. coli DNA Polymerase, followed by ligation; and in the second instance, by ligation alone.

Immunoprecipitation of the fusion proteins with a panel of monoclonal antibodies directed against CD4

20 epitopes showed that all of the epitopes were preserved. A specific high affinity association is demonstrated between the chimeric molecules and HIV envelope proteins expressed on the surface of cells transfected with an attenuated (reverse transcriptase deleted) proviral construct.

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Table 1

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                    F N                                         S                   B
                    N S               B         M         H     DHA                 S
                    U P               B         N         G     RAU                 T
                    4 B               V         L         A     AE9                 X
                    H 2               1         1         1     236                 1
                                                                  /
     GCCTGTTTGAGAAGCAGCGGGCAAGAAAGACGCAAGCCCAGAGGCCCTGCCATTTCTGTG
   1 --------+---------+---------+---------+---------+---------+ 60
     CGGACAAACTCTTCGTCGCCCGTTCTTTCTGCGTTCGGGTCTCCGGGACGGTAAAGACAC


            B       PS                    S                                     S
          DBS  ADNPA        D         DHNA                M     HM              HNC
          DAP  VRLUU        D         RALU                N     AN              PCR
          EN1  AAAM9        E         AEA9                L     EL              AIF
          122  22416        1         2346                1     31              211
            /    / //                 /                         /               /
     GGCTCAGGTCCCTACTGGCTCAGGCCCCTGCCTCCCTCGGCAAGGCCACAATGAACCGGG
  61 --------+---------+---------+---------+---------+---------+ 120
     CCGAGTCCAGGGATGACCGAGTCCGGGGACGGAGGGAGCCGTTCCGGTGTTACTTGGCCC


                                                                M   N   R   G  -
            H                                           F
            I               B         F         HH      N   M   D
            N               B         N         HA      U   N   D
            F               V         U         AE      4   L   E
            1               1         4         12      H   1   1
                                      H
     GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGGCTCCTCCCAGCAGCCACTC
 121 --------+---------+---------+---------+---------+---------+ 180
     CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


          V   P   F   R   H   L   L   L   V   L   Q   L   A   L   L   P   A   A   T   Q -
            B       E   E                                           R       A
            B       C   C                                           S       L
            V       0   0                                           A       U
            1       K   K                                           1       1
     AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
 181 --------+---------+---------+---------+---------+---------+ 240
     TCCCTTTCTTTCACCACGACCCGTTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA


          G   K   K   V   V   L   G   K   K   G   D   T   V   E   L   T   C   T   A   S -
```

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                              M  M                                          H
                              B  B                                          I
                              O  O                                          N
                              2  2                                          F
 5                                                                          1
           CCCAGAAGAAGAGCATACAATTCCACTGGAAAAAACTCCAACCAGATAAAGATTCTGGGAA
       241 -----------+-----------+-----------+-----------+-----------+ 300
           GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT
10
            Q  K  K  S  I  Q  F  H  W  K  N  S  N  Q  I  K  I  L  G  N -

                       B              S                 S     F  H
                       NBS      F     AA          A     A     N  H  I
15                     LAP      O     VU          L     U     U  H  N
                       AN1      K     A9          U     3     D  A  F
                       422      1     26          1     A     2  1  1
                                /           /
           ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
20     301 -----------+-----------+-----------+-----------+-----------+ 360
           TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT

            Q  G  S  F  L  T  K  G  P  S  K  L  N  D  R  A  D  S  R  R -

                     S                    S        H           H
25                   MANAS                BA       I     A      I  D
                     BVLUT                CU       N     F      N  D
                     OAA9Y                L3       F     L      F  E
                     22461                1A       1     2      1  1
                       /                    /
           GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
30     361 -----------+-----------+-----------+-----------+-----------+ 420
           CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA

            S  L  W  D  Q  G  N  F  P  L  I  I  K  N  L  K  I  E  D  S -

                                        S
35                M     M      AMAM                       M
                  B     N      VNUN                       A
                  O     L      AL9L                       E
40                2     1      2161                       1
                                //
           CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
       421 -----------+-----------+-----------+-----------+-----------+ 480
45         GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC

            D  T  Y  I  C  E  V  E  D  Q  K  E  E  V  Q  L  L  V  F  G -


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                                          B                            S
                                          S                            T
                                          P                            Y
                                          M                            1
                                          1
 5
     GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
 481 --------+---------+---------+---------+---------+---------+ 540
     CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC

10      L  T  A  N  S  D  T  H  L  L  Q  G  Q  S  L  T  L  T  L  E -


            B   BS                                H
           BS   SC              D           M     I     S
           AP   TR              D           N     N     T
           N1   NF              E           L     F     Y
15         22   11              1           1     1     1
            /    /
     AGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC
20 541 --------+---------+---------+---------+---------+---------+ 600
     TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTTGTATG

        S  P  P  G  S  S  P  S  V  Q  C  R  S  P  R  G  K  N  I  Q -

25                       N           BBH S   B           BS
                M   MD  ASP       A  BSSGSC  S   B  N     SC
                B   ND  LPV       L  APTIAR  T   A  L     TR
                0   LE  UBU       U  N1NACF  X   N  A     NF
                2   11  122       1  221111  1   1  4     11
30                       //          / ///           /
     AGGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT
601 --------+---------+---------+---------+---------+---------+ 660
     TCCCCCCCCTTCTGGGAGAGGCACAGAGTCGACCTCGAGGTCCTATCACCGTGGACCTGTA

35      G  G  K  T  L  S  V  S  Q  L  E  L  Q  D  S  G  T  W  T  C -

     N
     NS                             M                    NM  A
     LP                             B                    HA  L
40   AH                             0                    EE  U
     31                             2                    11  1
      /
     GCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAATAGACATCGTGGTGCTAGCTT
661 --------+---------+---------+---------+---------+---------+ 720
45   CGTGACAGAACGTCTTGGTCTTCTTCCACCTCAAGTTTTATCTGTAGCACCACGATCGAA

        T  V  L  Q  N  Q  K  K  V  E  F  K  I  D  I  V  V  L  A  F -

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```
                    HS              M    M
                    AT              N    N
                    EU              L    L
  5                 31              1    1
                     /
                    TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAGTTCTCCTTCC
               721  --------•---------•---------•---------•---------•---------• 780
                    AGGTCTTCCGGAGGTCGTATCAGATATTCTTTCTCCCCCTTGTCCACCTCAAGAGGAAGG
 10
                     Q  K  A  S  S  I  V  Y  K  K  E  G  E  Q  V  E  F  S  F  P  -

                                 A              A    M
                                 L              L    N
                                 U              U    L
 15                              1              1    1
                    CACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGGCAGGCGGAGA
               781  --------•---------•---------•---------•---------•---------• 840
                    GTGAGCGGAAATGTCAACTTTTCGACTGCCCGTCACCGCTCGACACCACCGTCCGCCTCT
 20
                     -L  A  F  T  V  E  K  L  T  G  S  G  E  L  W  W  Q  A  E  R  -

                                 P  S
                          H  M FM A                              M
 25                       P  N LN U                              B
                          H  L ML 3                              D
                          1  1 11 A                              2
                    GGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAAGTGTCTGTAA
               841  --------•---------•---------•---------•---------•---------• 900
                    CCCGAAGGAGGAGGTTCAGAACCTAGTGGAAACTGGACTTCTTGTTCCTTCACAGACATT
 30
                     A  S  S  S  K  S  W  I  T  F  D  L  K  N  K  E  V  S  V  K  -

                          B      BS   PS
 35                       SM     SCADNPAD   A              A H
                          TA     TRVRLUUD   L              L P
                          EE     NFAAAM9E   U              U H
                          23     11224161   1              1 1
                           /      / // //
                    AACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTCCACCTCACCC
               901  --------•---------•---------•---------•---------•---------• 960
                    TTGCCCAATGGGTCCTGGGATTCGAGGTCTACCCGTTCTTCGAGGGCGAGGTGGAGTGGG
 40
                     R  V  T  Q  D  P  K  L  Q  M  G  K  K  L  P  L  H  L  T  L  -
 45



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                         BS                                    BSS
          M              SC  HS          D           M    H    SCAHM
          N              TR  AT          D           N    P    TRUAN
          L              NF  EU          E           L    H    NF9EL
5         1              11  31          1           1    1    11631
                          /   /                                 /    /
          TGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCCCCTTGAAGCGA
     961  ------------◆----------◆----------◆----------◆----------◆ 1020
          ACGGGGTCCGGAACGGAGTCATACGACCGAGACCTTTGGAGTGGGACCGGGAACTTCGCT
10

            P   Q   A   L   P   Q   Y   A   G   S   G   N   L   T   L   A   L   E   A   K  -


                             S           BS
                             F           SC                      H  D    A
15                           A           TR                      P  D    L
                             N           NF                      H  E    U
                             1           11                      1  1    1

                                          /
          AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGA
20   1021 ------------◆----------◆----------◆----------◆----------◆ 1080
          TTTGTCCTTTCAACGTAGTCCTTCACTTGGACCACCACTACTCTCGGTGAGTCGAGGTCT

            T   G   K   L   H   Q   E   V   N   L   V   V   M   R   A   T   Q   L   Q   K  -


                                  PS         S
25                M               ADNNPA      DF   AM         DE   A
                  N               VRLLUU      DA   LN         DS   L
                  L               AAAAM9      EN   UL         EP   U
                  1               224416      11   11         11   1
                   /               /////       /    /          /
          AAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAAAC
30   1081 ------------◆----------◆----------◆----------◆----------◆ 1140
          TTTTAAACTGGACACTCCACACCCCTGGGTGGAGGGGATTCGACTACGACTCGAACTTTG

            N   L   T   C   E   V   W   G   P   T   S   P   K   L   M   L   S   L   K   L  -

35
                  M                 T           H           N          DW
                  N                 A           P           N          DS
                  L                 Q           A           L          ET
40                1                 1           2           1          12
                                                                        /
          TGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGCCGGTGTGGGTGCTGAACCCTG
     1141 ------------◆----------◆----------◆----------◆----------◆ 1200
          ACCTCTTGTTCCTCCGTTTCCAGAGCTTCGCCCTCTTCGGCCACACCCACGACTTGGGAC
45
            E   N   K   E   A   K   V   S   K   R   E   K   P   V   W   V   L   N   P   E  -
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                                H                    PS          H
                  F      D   M   I    A              ADPA        I
                  O      D   A   N    V              VRUU        N
                  K      E   E   F    A              AAM9        F
                  1      1   3   1    1              2216        1
                                                      ///

         AGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTGGAATCCAACA
    1201  --------+---------+---------+---------+---------+---------+  1260
         TCCGCCCCTACACCGTCACAGACGACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT

           A   G   M   W   Q   C   L   L   S   D   S   G   Q   V   L   L   E   S   N   I -

                  S          SA   BHF BS                        H
                 ANA        HNCP SGNMAANXA                      RSD  I  A
                 VLU        PCRA PIUNMULHV                      SCD  N  L
                 AA9        AIFL 1ADLH3ADA                      AAE  D  U
                 236        2111 21211A421                      111  3  1
                  //         //   / / /                          /

         TCAAGGTTCTGCCCACATGGTCCACCCCGGTGCACGCGGATCCCGAGGGTGAGTACTAAG
    1261  --------+---------+---------+---------+---------+---------+  1320
         AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCGCCTAGGGCTCCCACTCATGATTC

           K   V   L   P   T   W   S   T   P   V   H   A   D   P   E

                         BS                                                B
          H           H  SC  HS        S           M                   M   D   S
          P           A  TR  AT        T           N                   N   D   P
          H           E  NF  EU        Y           L                   L   D   M
          1           3  11  31        1           1                   1   E   1
          /              /   /         /           /                   /   1   1
                                                                       /   /   /
         CTTTCTGGGGCAGGCCAGGCCTGACCTTGGCTTTGGGGCAGGGAGGGGGGCTAAGGTGAGG
    1321  --------+---------+---------+---------+---------+---------+  1380
         GAAAGACCCCGTCCGGTCCGGACTGGAACCGAAACCCCGTCCCTCCCCCGATTCCACTCC

                  B                A    BH                        B      P
          BASHBHHNN   P     SG                          N      BS      F                  H
          AHPHBAPAL   A     PI                          L      AP      L                  G
          NAMAEEHRA   L     1A                          A      N1      M                  A
          121112114   1     21                          3      22      1                  1
            / ////          /                                  /
         CAGGTGGCGCCAGCAGGTGCACACCCAATGCCCATGAGCCCAGACACTGGACGCTGAACC
    1381  --------+---------+---------+---------+---------+---------+  1440
         GTCCACCGCGGTCGTCCACGTGTGGGTTACGGGTACTCGGGTCTGTGACCTGCGACTTGG

          F                    BS      S           B SS      B S             FN
          N          M         SC    DNHA        H SMAAHNABSAC              NS
          U          N         TR    RLAU        H TNUUALPAPLR              UP
          D          L         NF    AAE9        A NL99EAAN1UF              DB
          2          1         11    2436        1 11663412211             22
                                /      /           / / / /                  /
         TCGCGGACAGTTAAGAACCCAGGGGCCTCTGCGCCTGGGCCCAGCTCTGTCCCACACCGC
    1441  --------+---------+---------+---------+---------+---------+  1500
         AGCGCCTGTCAATTCTTGGGTCCCCGGAGACGCGGACCCGGGTCGAGACAGGGTGTGGCG
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17

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                              F                           BSS            BS
      MS        BNN           NM         S  BMDMHNABSAA              SCB
      AA        ALL           UN         T  BBRNALPAPUU              TRA
      EC        NAA           4L         Y  VOALEAAN199              NFN
      32        134           H1         1  12213412266              111
       /                       /          //  /////                   /
      GGTCACATGGCACCACCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGG
 1501 ---------+---------+---------+---------+---------+---------+ 1560
      CCAGTGTACCGTGGTGGAGAGAACGTCGGAGGTGGTTCCCGGGTAGCCAGAAGGGGGACC
                     A  S  T  K  G  P  S  V  F  P  L  A -

                    BH          B  NFS  BS   F   BS
      N         M   MSG        MSB SNAH  SC   N   SC
      L         N   NPI        NPB PUUA  TR   U   TR
      A         L   L1A        L1V B49E  NF   4   NF
      4         1   121        121 2H63  11   H   11
       /             /              /           /
      CACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
 1561 ---------+---------+---------+---------+---------+---------+ 1620
      GTGGGAGGAGGTTCTCGTGGAGACCCCCGTGTCGCCGGGACCCGACGGACCAGTTCCTGA
        P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y -

                                              NF   A  BH
             H  M     T      H     D  BANHBHN  SN   P  SG
             P  A     T      P     D  AHAHBAL  PU   A  PI
             A  E     H      H     E  NARAEEA  B4   L  1A
             2  3     1      1     1  1211124  2H   1  21
                                    /   //           /
      ACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACA
 1621 ---------+---------+---------+---------+---------+---------+ 1680
      TGAAGGGGCTTGGCCACTGCCACAGCACCTTGAGTCCGCGGGACTGGTCGCCGCACGTGT
       F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H  T -

             S                 H              F     B
      HNC              DM    I  M        D    N  M  SM  B
      PCR              DS    N  N        D    U  N  TA  B
      AIF              ET    F  L        E    4  L  EE  V
      211              12    1  1        1    H  1  23  1
       //               /
      CCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGC
 1681 ---------+---------+---------+---------+---------+---------+ 1740
      GGAAGGGCCGACAGGATGTCAGGAGTCCTGAGATGAGGGAGTCGTCGCACCACTGGCACG
       F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V  P -

      B    F  B     B                        H
      SH   N  ASM B NSB                  M    I
      PP   U  LTN A LPB                  A    N
      1H   4  UXL N A1V                  E    F
      21   H  111 1 421                  2    1
       /
      CCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA
 1741 ---------+---------+---------+---------+---------+---------+ 1800
      GGAGGTCGTCGAACCCGTGGGTCTGGATGTAGACGTTGCACTTAGTGTTCGGGTCGTTGT
        S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S  N  T -
```

```
          S                   M                   HM    HM
          T                   N                   AN    PN
          Y                   L                   EL    HL
          1                   1                   31    11
          CCAAGGTGGACAAGAAAGTTGGTGAGAGGCCAGCACAGGGAGGGAGGGTGTCTGCTGGAA
     1801 --------------------------------------------------------------+ 1860
          GGTTCCACCTGTTCTTTCAACCACTCTCCGGTCGTGTCCCTCCCTCCCACAGACGACCTT


          K   V   D   K   K   V

                  E           BS              SS        F          BS    F
          DE      CHH  F      SC              HHNCF     N          BSC   N
          DS      0HA  0      TR              PGCRA     U          BTR   U
          EP      4AE  K      NF              AAIFN     4          VNF   4
          11      712  1      11              21111     H          111   H

          GCAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGCA
     1861 --------------------------------------------------------------+ 1920
          CGTCCGAGTCGCGAGGACGGACCTGCGTAGGGCCGATACGTCGGGGTCAGGTCCCGTCGT


                  S              S
          DBHMHNA        HMNCN       M      MNDM
          RBABPLU        PNCRL       N      NLDB
          AVEOHA9        ALIFA       L      LAEO
          2132146        21114       1      1312

          AGGCAGGCCCCGTCTGCCTCTTCACCCGGAGCCTCTGCCCGCCCCACTCATGCTCAGGGA
     1921 --------------------------------------------------------------+ 1980
          TCCGTCCGGGGCAGACGGAGAAGTGGGCCTCGGAGACGGGCGGGGTGAGTACGAGTCCCT


                          BS   P                      B        BS
                          SC   F              M  B N  S        SC
                          TR   L              A  A L  P        TR
                          NF   M              E  N A  1        NF
                          11   1              1  1 4  2        11

          GAGGGTCTTCTGGCTTTTTCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCA
     1981 --------------------------------------------------------------+ 2040
          CTCCCAGAAGACCGAAAAAGGGTCCGAGACCCGTCCGTGTCCGATCCACGGGGATTGGGT


          S            B              B        B        S
          DHA          S              DBS      S   M    HNC   A
          RAU          P              DAP      P   N    PCR   V
          AE9          M              EN1      M   L    AIF   A
          236          1              122      1   1    211   2

          GGCCCTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAG
     2041 --------------------------------------------------------------+ 2100
          CCGGGACGTGTGTTTCCCCGTCCACGACCCGAGTCTGGACGGTTCTCGGTATAGGCCCTC
```

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```
        PS
        DNPA          D                    H                        D    A    M
        RLUU          D                    A                        D    L    N
        AAM9          E                    E                        E    U    L
        2416          1                    3                        1    1    1
        / //
        GACCCTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGA
 2101   --------+---------+---------+---------+---------+---------+   2160
        CTGGGACGGGGACTGGATTCGGGTGGGGGTTTCCGGTTTGAGAGGTGAGGGAGTCGAGCCT


                      H                                                  B
                      I    M  MM                                 P       BS
                      N    N  AB                                 S       AP
                      F    L  EO                                 T       N1
                      1    1  32                                 1       22
                               /                                          /
        CACCTTCTCTCCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGAGCCCAAATCT
 2161   --------+---------+---------+---------+---------+---------+   2220
        GTGGAAGAGAGGAGGGTCTAAGGTCATTGAGGGTTAGAAGAGAGACGTCTCGGGTTTAGA
                                                             E    P    K    S    -


                      N              BBS              BS
        M             NS             SSC              SC  HS                  M
        A             LP             PTR              TR  AT                  N
        E             AH             1NF              NF  EU                  L
        3             31             211              11  31                  1
                                     /                /   /
        TGTGACAAAACTCACACATGCCCACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCC
 2221   --------+---------+---------+---------+---------+---------+   2280
        ACACTGTTTTGAGTGTGTACGGGTGGCACGGGTCCATTCGGTCGGGTCCGGAGCGGGAGG
        C  D  K  T  H  T  C  P  P  C  P


                            B                    BS  S         S            S
        A    M         B N  SM F                  SC  F     DHNA      HNC
        L    N         A L  PA 0                  TR  A     RALU      PCR
        U    L         N A  1E K                  NF  N     AEA9      AIF
        1    1         1 4  21 1                  11  1     2346      211
                                                  /         /          /
        AGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGG
 2281   --------+---------+---------+---------+---------+---------+   2340
        TCGAGTTCCGCCCTGTCCACGGGATCTCATCGGACGTAGGTCCCTGTCCGGGGTCGGCCC


                                                  BS        S
        A M  M                  M D          M    SC  M ANA M
        F A  B                  N D          N    TR  B VLU B
        L E  0                  L E          L    NF  0 AA9 0
        3 2  2                  1 1          1    11  2 246 2
                                                  /     /
        TGCTGACACGTCCACCTCCATCTCTTCCTCAGCACCTGAACTCCTGGGGGGACCGTCAGT
 2341   --------+---------+---------+---------+---------+---------+   2400
        ACGACTGTGCAGGTGGAGGTAGAGAAGGAGTCGTGGACTTGAGGACCCCCCTGGCAGTCA
                            A  P  E  L  L  G  G  P  S  V  -
```

```
                                                S              SS
                       M           S            AN     M  HMANNAC  DM    M
                       N           T            UL     N  PNVCLUR  DS    A
                       L           Y            3A     L  ALAIA9F  ET    E
                       1           1            A3     1  2121461  12    3
                                                 /      /  / //     /
        CTTCCTCTTCCCCCCAAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC
2401    ---------+---------+---------+---------+---------+---------+    2460
        GAAGGAGAAGGGGGGTTTTGGGTTCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAGTG

         F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  -

            N
            NS          M           M           DM    M              RM    M
            LP          A           N           DS    B              SA    N
            AH          E           L           ET    0              AE    L
            31          2           1           12    2              12    1
             /                                   /
        ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGA
2461    ---------+---------+---------+---------+---------+---------+    2520
        TACGCACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCT

         C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  -

                                     F  FN
                          M     N   NSS                R           M   R
                          N     U   UPA                S           A   S
                          L     4   DBC                A           E   A
                          1     H   222                1           2   1
                                     //
        CGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA
2521    ---------+---------+---------+---------+---------+---------+    2580
        GCCGCACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGCAT

         G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  -

            S                         BS
         HNC   HH               M     SC                        R
         PCR   GP               N     TR                        S
         AIF   AH               L     NF                        A
         211   11               1     11                        1
          /                           /
        CCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
2581    ---------+---------+---------+---------+---------+---------+    2640
        GGCCCACCAGTCGCAGGAGTGGCAGGACGTGGTCCTGACCGACTTACCGTTCCTCATGTT

         R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  -

                                     M     T
                                     N     A
                                     L     Q
                                     1     1
        GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA
2641    ---------+---------+---------+---------+---------+---------+    2700
        CACGTTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTT
         C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  -
```

```
              P S              S                        S
              ADNNPMA          A H M     N       HHN    BSAH
              VRLLUNU          U A N     L       APA    GFUA
              AAAAML9          9 E L     A       EAE    LI9E
              2244116          6 3 1     3       321    1163
              ///// /                                   /
              AGGTGGGACCCGTGGGGTGCGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTG
      2701    ------------+---------+---------+---------+---------+---------+  2760
              TCCACCCTGGGCACCCCACGCTCCCGGTGTACCTGTCTCCGGCCGAGCCGGGTGGGAGAC

                              N                        F
              D M   M         S     R                  N     A        B
              D N   A         P     S                  U     V        B
              E L   E         B     A                  4     A        V
              1 1   3         2     1                  H     1        1
                                          M
                                          N
                                          L
                                          1
              CCCTGAGAGTGACCGCTGTACCAACCTCTGTCCTACAGGGCAGCCCCGAGAACCACAGGT
      2761    ------------+---------+---------+---------+---------+---------+  2820
              GGGACTCTCACTGGCGACATGGTTGGAGACAGGATGTCCCGTCGGGGCTCTTGGTGTCCA

                                                 G  Q  P  R  E  P  Q  V  -

                            SS              BS                      BS
              R F           AHNNCCS   A   F  SC                      SC
              S 0           VPCCRRM   L   0  TR                      TR
              A K           AAIIFFA   U   K  NF                      NF
              1 1     .     1211111   1   1  11                      11
                            /////                /                        /
              GTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCT
      2821    ------------+---------+---------+---------+---------+---------+  2880
              CATGTGGGACGGGGGTAGGGCCCTACTCGACTGGTTCTTGGTCCAGTCGGACTGGACGGA

                 Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  -

                 B                                               F
                 S                                               N     H
                 P                                               U     P
                 M                                               4     A
                 1                                               H     2
              GGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGA
      2881    ------------+---------+---------+---------+---------+---------+  2940
              CCAGTTTCCGAAGATAGGGTCGCTGTAGCGGCACCTCACCCTCTCGTTACCCGTCGGCCT

                 V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  -

                                           H
                 B                         M I   M       N           H
                 B                         N N   B       L           P
                 V                         L N   0       A           H
                 1                         1 F   2       4           1
                                             1
              GAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG
      2941    ------------+---------+---------+---------+---------+---------+  3000
              CTTGTTGATGTTCTGGTGCGGAGGGCACGACCTGAGGCTGCCGAGGAAGAAGGAGATGTC

                 N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  -
```

```
                    B           F                       S
      M A           S          NM         MBX          NF  M
      N L           P          UB         ABM          LA  N
      L U           M          40         EVN          AN  L
      1 1           1          H2         211          31  1
                                                        /
     CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGAT
3001 ------------+-----------+-----------+-----------+-----------+-----------+ 3060
     GTTCGAGTGGCACCTGTTCTCGTCCACCGTCGTCCCCTTGCAGAAGAGTACGAGGCACTA

      K L T V D K S R W Q Q G N V F S C S V M -
```

```
                                                            S
      N N                                  M   M   HNC
      S L                                  B   N   PCR
      I A                                  0   L   AIF
      1 3                                  2   1   211
                                                    /
     GCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG
3061 ------------+-----------+-----------+-----------+-----------+-----------+ 3120
     CGTACTCCGAGACGTGTTGGTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCATTTAC

      H E A L H N H Y T Q K S L S L S P G K .
```

```
      CXHHN
      FMAPA
      RAEAE
      13321
          /
     AGTGCGACGGCCG
3121 ------------+--- 3133
     TCACGCTGCCGGC
```

23

Table 2

```
     F N                                         S                        B
     N S                  B        M        H    DHA                      S
     U P                  B        N        G    RAU                      T
     4 B                  V        L        A    AE9                      X
     H 2                  1        1        1    236                      1
                                                   /
  GCCTGTTTGAGAAGCAGCGGGCAAGAAAGACGCAAGCCCAGAGGCCCTGCCATTTCTGTG
1 --------+---------+---------+---------+---------+---------+ 60
  CGGACAAACTCTTCGTCGCCCGTTCTTTCTGCGTTCGGGTCTCCGGGACGGTAAAGACAC


     B     PS                        S                                    S
   DBS  ADNPA           D        DHNA                 M    HM             HNC
   DAP  VRLUU           D        RALU                 N    AN             PCR
   EN1  AAAM9           E        AEA9                 L    EL             AIF
   122  22416           1        2346                 1    31             211
     /   / //                    /                         /                /
  GGCTCAGGTCCCTACTGGCTCAGGCCCCTGCCTCCCTCGGCAAGGCCACAATGAACCGGG
61 --------+---------+---------+---------+---------+---------+ 120
  CCGAGTCCAGGGATGACCGAGTCCGGGGACGGAGGGAGCCGTTCCGGTGTTACTTGGCCC


                                                   M   N    R    G  -

   H                             F                 F
   I                  B          N        HH       N   M    D
   N                  B          U        HA       U   N    D
   F                  V          4        AE       4   L    E
   1                  1          H        12       H   1    1
  GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
121 --------+---------+---------+---------+---------+---------+ 180
  CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


      V  P  F  R  H  L  L  L  V  L  Q  L  A  L  L  P  A  A  T  Q  -

         B     E  E                                      R       A
         B     C  C                                      S       L
         V     O  O                                      A       U
         1     K  K                                      1       1
  AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
181 --------+---------+---------+---------+---------+---------+ 240
  TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA


      G  K  K  V  V  L  G  K  K  G  D  T  V  E  L  T  C  T  A  S  -

                                                         H
                    M  M                                 I
                    B  B                                 N
                    O  O                                 F
                    2  2                                 1
  CCCAGAAGAAGAGCATACAATTCCACTGGAAAAAACTCCAACCAGATAAAGATTCTGGGAA
241 --------+---------+---------+---------+---------+---------+ 300
  GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTTGAGGTTGGTCTATTTCTAAGACCCTT


      Q  K  K  S  I  Q  F  H  W  K  N  S  N  Q  I  K  I  L  G  N  -
```

```
                    B                       S                       S       F    H
                  NBS           F          AA            A          AU3A   NUD2  HINF1
                  LAP           OK          VU            LU1        ...    HHA1
                  AN1                       A9
                  422                       26
                   /                         /
          ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
      301 ---------+---------+---------+---------+---------+---------+ 360
          TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT
            Q   G   S   F   L   T   K   G   P   S   K   L   N   D   R   A   D   S   R   R -


                       S                       S            H          H
                     MANAS                     BA           INF        A          HINF1   DDE1
                     BVLUT                     CUL3         1           FL2        NF1
                     DAA9Y                     1A
                     22461
                       /                         /
          GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
      361 ---------+---------+---------+---------+---------+---------+ 420
          CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA
            S   L   W   D   Q   G   N   F   P   L   I   I   K   N   L   K   I   E   D   S -


                     M            M            S                                  M
                     BO2          MNL1         AMAM                               AE1
                                               VNUN
                                               AL9L
                                               2161
                                                 //
          CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
      421 ---------+---------+---------+---------+---------+---------+ 480
          GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC
            D   T   Y   I   C   E   V   E   D   Q   K   E   E   V   Q   L   L   V   F   G -


                                               B                                  S
                                               SPM1                               TY1

          GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
      481 ---------+---------+---------+---------+---------+---------+ 540
          CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC
            L   T   A   N   S   D   T   H   L   L   Q   G   Q   S   L   T   L   T   L   E -


                     B    BS                                    H
                     BS   SC                 D            M      INF        S
                     AP   TR                 DDE1         MNL1   1          STY1
                     N1   NF
                     22   11
                      /    /
          AGAGCCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC
      541 ---------+---------+---------+---------+---------+---------+ 600
          TCTCGGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTTGTATG
            S   P   P   G   S   S   P   S   V   Q   C   R   S   P   R   G   K   N   I   Q -
```

25

```
                              N         BBH S  B         BS
                M    MD     ASP    A BSSGSC  S    B  N    SC
                B    ND     LPV    L APTIAR  T    A  L    TR
                0    LE     UBU    U N1NACF  X    N  A    NF
                2    11     122    1 221111  1    1  4    11
                              //        / ///               /
        AGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT
   601  -------------+---------+---------+---------+---------+---------+ 660
        TCCCCCCCTTCTGGGAGAGGCACAGAGTCGACCTCGAGGTCCTATCACCGTGGACCTGTA
          G   G   K   T   L   S   V   S   Q   L   E   L   Q   D   S   G   T   W   T   C  -

          N
          NS                                    M                      NM   A
          LP                                    B                      HA   L
          AH                                    0                      EE   U
          31                                    2                      11   1
          /
        GCACTGTCTTGCAGAACCAGAAGAAGGTGGACTTCAAAATAGACATCGTGGTGCTAGCTT
   661  -------------+---------+---------+---------+---------+---------+ 720
        CGTGACAGAACGTCTTGGTCTTCTTCCACCTGAAGTTTTATCTGTAGCACCACGATCGAA
          T   V   L   Q   N   Q   K   K   V   E   F   K   I   D   I   V   V   L   A   F  -

                HS        M   M
                AT        N   N
                EU        L   L
                31        1   1
                /
        TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGGAACAGGTGGAGTTCTCCTTCC
   721  -------------+---------+---------+---------+---------+---------+ 780
        AGGTCTTCCGGAGGTCGTATCAGATATTCTTTCTCCCCCCTTGTCCACCTCAAGAGGAAGG
            Q   K   A   S   S   I   V   Y   K   K   E   G   E   Q   V   E   F   S   F   P  -

                          A               A       M
                          L               L       N
                          U               U       L
                          1               1       1
        CACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGGCAGGCGGAGA
   781  -------------+---------+---------+---------+---------+---------+ 840
        GTGAGCGGAAATGTCAACTTTTCGACTGCCCGTCACCGCTCGACACCACCGTCCGCCTCT
            L   A   F   T   V   E   K   L   T   G   S   G   E   L   W   W   Q   A   E   R  -

                          P   S
                H    M   FV   A                            M
                P    N   LN   U                            B
                H    L   ML   3                            0
                1    1   11   A                            2
        GGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAAGTGTCTGTAA
   841  ----+---------+---------+---------+---------+---------+---------+ 900
        CCCGAAGGAGGAGGTTCAGAACCTAGTGGAAACTGGACTTCTTGTTCCTTCACAGACATT
            A   S   S   S   K   S   W   I   T   F   D   L   K   N   K   E   V   S   V   K  -
```

```
        B          BS    PS
        SM         SCADNPAD   A                    A H
        TA         TRVRLUUD   L                    L P
        EE         NFAAAM9E   U                    U H
        23         11224161   1                    1 1
         /          / / //
       AACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTCCACCTCACCC
   901 -----------+----------+----------+----------+----------+----------+ 960
       TTGCCCAATGGGTCCTGGGATTCGAGGTCTACCCGTTCTTCGAGGGCGAGGTGGAGTGGG
         R  V  T  Q  D  P  K  L  Q  M  G  K  K  L  P  L  H  L  T  L -


            BS                                       BSS
       M    SC HS    D              M  H             SCAHM
       N    TR AT    D              N  P             TRUAN
       L    NF EU    E              L  H             NF9EL
       1    11 31    1              1  1             11631
             /  /                                     / /
       TGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCCCTTGAAGCGA
   961 -----------+----------+----------+----------+----------+----------+ 1020
       ACGGGGTCCGGAACGGAGTCATACGACCGAGACCTTTGGAGTGGGACCGGGAACTTCGCT
         P  Q  A  L  P  Q  Y  A  G  S  G  N  L  T  L  A  L  E  A  K -


                   S            BS
                   F            SC              H D    A
                   A            TR              P D    L
                   N            NF              H E    U
                   1            11              1 1    1
                                 /
       AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGA
  1021 -----------+----------+----------+----------+----------+----------+ 1080
       TTTGTCCTTTCAACGTAGTCCTTCACTTGGACCACCACTACTCTCGGTGAGTCGAGGTCT
         T  G  K  L  H  Q  E  V  N  L  V  V  M  R  A  T  Q  L  Q  K -


                         PS             S
       N                 ADNNPA         DF  AM       DE  A
       N                 VRLLUU         DA  LN       DS  L
       L                 AAAAM9         EN  UL       EP  U
       1                 224416         11  11       11  1
                          /////          /   /        /
       AAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAAAC
  1081 -----------+----------+----------+----------+----------+----------+ 1140
       TTTTAAACTGGACACTCCACACCCCTGGGTGGAGGGGATTCGACTACGACTCGAACTTTG
         N  L  T  C  E  V  W  G  P  T  S  P  K  L  M  L  S  L  K  L -


       M                        T             H          M       DM
       N                        A             P          N       DS
       L                        Q             A          L       ET
       1                        1             2          1       12
                                                                  /
       TGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGCCGGTGTGGGTGCTGAACCCTG
  1141 -----------+----------+----------+----------+----------+----------+ 1200
       ACCTCTTGTTCCTCCGTTTCCAGAGCTTCGCCCTCTTCGGCCACACCCACGACTTGGGAC
         E  N  K  E  A  K  V  S  K  R  E  K  P  V  W  V  L  N  P  E -
```

27

<pre>
                                    H   PS   H
                         F   D   M   I A   ADPA   I
                         0   D   A   N V   VRUU   N
                         K   E   E   F A   AAM9   F
                         1   1   3   1 1   2216   1
                                                     ///
           AGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTGGAATCCAACA
     1201 --------+---------+---------+---------+---------+---------+ 1260
           TCCGCCCCTACACCGTCACAGACGACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT

             A   G   M   W   Q   C   L   L   S   D   S   G   Q   V   L   L   E   S   N   I-

                                 S   SA   BHF BS   H
                               ANA   HNCP   SGNMAANXA   RSD I A
                               VLU   PCRA   PIUNMULHV   SCD N L
                               AA9   AIFL   1ADLH3AOA   AAE D U
                               236   2111   21211A421   111 3 1
                                //   //   / / / /   /
           TCAAGGTTCTGCCCACATGGTCCACCCCGGTGCACGCGGATCCCGAGGGTGAGTACTAAG
     1261 --------+---------+---------+---------+---------+---------+ 1320
           AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCGCCTAGGGCTCCCACTCATGATTC

             K   V   L   P   T   W   S   T   P   V   H   A   D   P   E

                     E   BS   SS   F   BS   F
                 H   CHH   F   SC   HHNCF   ·N   BSC N
                 P   OHA   D   TR   PGCRA   U   BTR U
                 H   4AE   K   NF   AAIFN   4   VNF 4
                 1   712   1   11   21111   H   111 H
                 /   /   /   //   //
           CTTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGCAAGGC
     1321 --------+---------+---------+---------+---------+---------+ 1350
           GAAGTCGCGAGGACGGACCTGCGTAGGGCCGATACGTCGGGGTCAGGTCCCGTCGTTCCG

                    S   S
              DBHVHNA   HVNCN   M   MNDM
              RB4SPLU   PNCRL   N   NLDB
              AVEOHA9   A_IFA   L   LAEO
              2132146   21114   1   1312
               // //   //
           ACGCCCCGTCTGCCTCTTCACCCGGAGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGG
     1381 --------+---------+---------+---------+---------+---------+ 1440
           TCCGGGGCAGACGGAGAAGTGGGCCTCGGAGACGGGCGGGGTGAGTACGAGTCCCTCTCC

                    BS   P   B   BS   S
                    SC   F   M   B   N   S   SCDHA
                    TR   L   A   A   L   P   TRRAU
                    NF   M   E   L   A   1   NFAE9
                    11   1   1   1   4   2   11236
                     /   / /
           GTCTTCTGGCTTTTTCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCC
     1441 --------+---------+---------+---------+---------+---------+ 1500
           CAGAAGACCGAAAAAGGGTCCCGAGACCCGTCCGTGTCCGATCCACGGGGATTGGGTCCGG
</pre>

28

```
                 B                    B            B          S        PS
                 S                   DBS           S    M     HNC      ADNPA
                 P                   DAP           P    N     PCR      VRLUU
                 M                   EN1           M    L     AIF      AAAM9
                 1                   122           1    1     211      22416
                                      /                        /        / //
      CTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACC
 1501 ---------+---------+---------+---------+---------+---------+ 1560
      GACGTGTGTTTCCCCGTCCACGACCCGAGTCTGGACGGTTCTCGGTATAGGCCCTCCTGG


                 D                    H             D    A     M
                 D                    A             D    L     N
                 E                    E             E    U     L
                 1                    3             1    1     1
      CTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGACACC
 1561 ---------+---------+---------+---------+---------+---------+ 1620
      GACGGGGACTGGATTCGGGTGGGGTTTCCGGTTTGAGAGGTGAGGGAGTCGAGCCTGTGG


            H
            I     M   MM                               P     BS          M
            N     N   AB                               S     AP          A
            F     L   EO                               T     N1          E
            1     1   32                               1     22          3
                       /                                      /
      TTCTCTCCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGAGCCCAAATCTTGTG
 1621 ---------+---------+---------+---------+---------+---------+ 1680
      AAGAGAGGAGGGTCTAAGGTCATTGAGGGTTAGAAGAGAGACGTCTCGGGTTTAGAACAC


                                                       E  P  K  S  C  D  -

                 N          BBS             BS
                 NS         SSC             SC  HS              M  A
                 LP         PTR             TR  AT              N  L
                 AH         1NF             NF  EU              L  U
                 3?         2?1             11  31              1  1
                  /          /               /  /
      ACAAAA?TCACACAT GCCCA??GTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCT
 1681 ---------+---------+---------+---------+---------+---------+ 1740
      TGTTTTGAGTGTGTACGGGTGGCACGGGTCCATTCGGTCGGGTCCGGAGCGGGAGGTCGA


         K  T  H  T  C  P  P  C  P

                              B                  BS   S         S         S
            M          B N   SN  F               SC   F      DHNA      HNC
            N          A L   PA  0               TR   A      RALU      PCR
            L          N A   1E  K               NF   N      AEA9      AIF
            1          1 4   21  1               11   1      2346      211
                                                  /            /         /
      CAAGGCGGGACAGGTGCCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCT
 1741 ---------+---------+---------+---------+---------+---------+ 1800
      GTTCCGCCCTGTCCACGGGATCTCATCGGACGTAGGTCCCTGTCCGGGGTCGGCCCACGA
```

```
                                                   BS        S
       A M  M              M  D          M .       SC  M ANA M
       F A  B              N  D          N         TR  B VLU B
       L E  0              L  E          L         NF  0 AA9 0
5      3 2  2              1  1          1         11  2 246 2
                                                    /         /
       GACACGTCCACCTCCATCTCTTCCTCAGCACCTGAACTCCTGGGGGGGACCGTCAGTCTTC
1801   --------+---------+---------+---------+---------+---------+   1860
       CTGTGCAGGTGGAGGTAGAGAAGGAGTCGTGGACTTGAGGACCCCCCTGGCAGTCAGAAG
10

                           A  P  E  L  L  G  G  P  S  V  F  -

                                   S               SS            N
             M         S           AN   M HMANNAC DM  M          NS
15           N         T           UL   N PNVCLUR DS  A          LP
             L         Y           3A   L ALAIA9F ET  E          AH
             1         1           A3   1 2121461 12  3          31
                                    /   / //      /              /
       CTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
20   1861  --------+---------+---------+---------+---------+---------+   1920
       GAGAAGGGGGGTTTTGGGTTCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAGTGTACG

       L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  -

             M         M          DM  M                RM  M
25           A         N          DS  B                SA  N
             E         L          ET  0                AE  L
             2         1          12  2                12  1
                                   /
       GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
30   1921  --------+---------+---------+---------+---------+---------+   1980
       CACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCG

       V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  -

                               F  FN                            S
                   N   N  NSS              R           M  R  HNC
35                 N   U  UPA              S           A  S  PCR
                   L   4  DBC              A           E  A  AIF
40                 1   H  222              1           2  1  211
                         //                              /
       GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGG
     1981  --------+---------+---------+---------+---------+---------+   2040
       CACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGCATGGCC
45
       V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  -
```

```
                                        BS
        HH                      M       SC                              R
        GP                      N       TR                              S
        AH                      L       NF                              A
        11                      1       11                              1
                                            /
        GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
2041    ---------+---------+---------+---------+---------+---------+    2100
        CACCAGTCGCAGGAGTGGCAGGACGTGGTCCTGACCGACTTACCGTTCCTCATGTTCACG

        V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  -


                                M       T
                                N       A
                                L       Q
                                1       1
        AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGT
2101    ---------+---------+---------+---------+---------+---------+    2160
        TTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCA

        K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K


            P S                 S                           S
        ADNNPMA                 A H M      N        HHN     BSAH         D
        VRLLUNU                 U A N      L        APA     GFUA         D
        AAAAML9                 9 E L      A        EAE     LI9E         E
        2244116                 6 3 1      3        321     1163         1
        /////  /                                           /
        GGGACCCGTGGGGTGCGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTGCCCT
2161    ---------+---------+---------+---------+---------+---------+    2220
        CCCTGGGCACCCCACGCTCCCGGTGTACCTGTCTCCGGCCGAGCCGGGTGGGAGACGGGA


                N
        M M     S     R                   M     F        A        B         R F
        N A     P     S                   N     N        V        B         S 0
        L E     B     A                   L     U        A        V         A K
        1 3     2     1                   1     4        1        1         1 1
                                                H
        GAGAGTGACCGCTGTACCAACCTCTGTCCTACAGGGCAGCCCCGAGAACCACAGGTGTAC
2221    ---------+---------+---------+---------+---------+---------+    2280
        CTCTCACTGGCGACATGGTTGGAGACAGGATGTCCCGTCGGGGCTCTTGGTGTCCACATG

                                G  Q  P  R  E  P  Q  V  Y  -
```

31

```
                  SS                      BS              BS B
              AHNNCCS      A       F       SC              SC S
              VPCCPRW      L       D       TR              TR P
              AAIIFFA      U       K       NF              NF M
              1211111      1       1       11              11 1
               //////                      /               /
       ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
  2281 --------+---------+---------+---------+---------+---------+ 2340
       TGGGACGGGGGTAGGGCCCTACTCGACTGGTTCTTGGTCCAGTCGGACTGGACGGACCAG

        T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  -

                                      F
                                      N       H                 B
                                      U       P                 B
                                      4       A                 V
                                      H       2                 1
       AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
  2341 --------+---------+---------+---------+---------+---------+ 2400
       TTTCCGAAGATAGGGTCGCTGTAGCGGCACCTCACCCTCTCGTTACCCGTCGGCCTCTTG

        K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  -

                                 H
                                 I
                             M   I   M           N           H       M  A
                             N   N   B           L           P       N  L
                             L   F   D           A           H       L  U
                             1   1   2           4           1       1  1
       AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAG
  2401 --------+---------+---------+---------+---------+---------+ 2460
       TTGATGTTCTGGTGCGGAGGGCACGACCTGAGGCTGCCGAGGAAGAAGGAGATGTCGTTC

        N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  -

                     B               F           MBX         S
                     S               NM          ABM         NF  M       N
                     P               UB          EVN         LA  N       S
                     M               40          211         AN  L       I
                     1               H2                      31  1       1
                                                              /
       CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
  2461 --------+---------+---------+---------+---------+---------+ 2520
       GAGTGGCACCTGTTCTCGTCCACCGTCGTCCCCTTGCAGAAGAGTACGAGGCACTACGTA

        L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  -
```

```
                                                          S
       N                                         M   M  HNC
       L                                         B   N  PCR
       A                                         D   L  AIF
       3                                         2   1  211
                                                          /
       GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTG
2521   ------------◆-----------◆------------◆---------------------◆------------◆ 2580
       CTCCGAGACGTGTTGGTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCATTTACTCAC

       E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   K   •

           CXH
           FMA
           RAE
           133
           /
       CGACGGCCG
2581   --------- 2589
       GCTGCCGGC
```

Table 3

```
                F N                                    S              B
                N S              B      M       H     DHA             S
                U P              B      N       G     RAU             T
                4 B              V      L       A     AE9             X
                H 2              1      1       1     236             1
                                                       /
        GCCTGTTTGAGAAGCAGCGGGCAAGAAAGACGCAAGCCCAGAGGCCCTGCCATTTCTGTG
      1 ---------+---------+---------+---------+---------+---------+ 60
        CGGACAAACTCTTCGTCGCCCGTTCTTTCTGCGTTCGGGTCTCCGGGACGGTAAAGACAC


          B       PS                  S                                  S
        DBS  ADNPA          D      DHNA                 M      HM       HNC
        DAP  VRLUU          D      RALU                 N      AN       PCR
        EN1  AAAW9          E      AEA9                 L      EL       AIF
        122  22416          1      2346                 1      31       211
          /   / //                   /                         /          /
        GGCTCAGGTCCCTACTGGCTCAGGCCCCTGCCTCCCTCGGCAAGGCCACAATGAACCGGG
     61 ---------+---------+---------+---------+---------+---------+ 120
        CCGAGTCCAGGGATGACCGAGTCCGGGGACGGAGGGAGCCGTTCCGGTGTTACTTGGCCC


                                                           M  N  R  G -
          H                        F                     F
          I              B         N              HH      N  M    D
          N              B         U              HA      U  N    D
          F              V         4              AE      4  L    E
          1              1         H              12      H  1    1
        GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
    121 ---------+---------+---------+---------+---------+---------+ 180
        CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


            V  P  F  R  H  L  L  L  V  L  Q  L  A  L  L  P  A  A  T  Q -
            B     E  E                                      R     A
            B     C  C                                      S     L
            V     0  0                                      A     U
            1     K  K                                      1     1
        AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
    181 ---------+---------+---------+---------+---------+---------+ 240
        TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA

            G  K  K  V  V  L  G  K  K  G  D  T  V  E  L  T  C  T  A  S -
```

```
          M    M                                                  H
          B    B                                                  I
          0    0                                                  N
          2    2                                                  F
                                                                  1
     CCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAA
241  ------------+----------+----------+----------+----------+    300
     GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT

       Q   K   K   S   I   Q   F   H   W   K   N   S   N   Q   I   K   I   L   G   N -


             B                 S                   S    F    H
            NBS      F        AA         A         AU   N  H  I
            LAP      0        VU         L         U    U  H  N
            AN1      K        A9         U         3    D  H  F
            422      1        26         1         A    2  A  1
                /                /                           1
     ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
301  ------------+----------+----------+----------+----------+    360
     TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT

       Q   G   S   F   L   T   K   G   P   S   K   L   N   D   R   A   D   S   R   R -


             S                      S    H         H
            MANAS     BA            BA   I    A    I    D
            BVLUT     CU            CU   N    F    N    D
            0AA9Y     L3            L3   F    L    F    E
            22461     1A            1A   1    2    1    1
                /                 /
     GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
361  ------------+----------+----------+----------+----------+    420
     CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA

       S   L   W   D   Q   G   N   F   P   L   I   I   K   N   L   K   I   E   D   S -


             M        M        S                      M
             B        N       AMAM                    A
             0        L       VNUN                    E
             2        1       AL9L                    1
                              2161
                               //
     CAGATACTTACATCTGTGAAGTGGACGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
421  ------------+----------+----------+----------+----------+    480
     GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC

       D   T   Y   I   C   E   V   E   D   Q   K   E   E   V   Q   L   L   V   F   G -
```

```
                                            B                               S
                                            S                               T
                                            P                               Y
                                            M                               1
                                            1
  5
        GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
    481 --------------+-------------+-------------+-------------+-------------+-------------+ 540
        CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC
 10
          L  T  A  N  S  D  T  H  L  L  Q  G  Q  S  L  T  L  T  L  E -

              B   BS                                   H
              BS  SC                 D           N     I        S
              AP  TR                 D           N     N        T
              N1  NF                 E           L     F        Y
 15           22  11                 1           1     1        1
              /   /
        AGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGGTAAAAACATAC
    541 --------------+-------------+-------------+-------------+-------------+-------------+ 600
 20     TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTTGTATG

          S  P  P  G  S  S  P  S  V  Q  C  R  S  P  R  G  K  N  I  Q -

                                 N             BBH S   B              BS
                     M   MD      ASP       A   BSSGSC  S   B   N      SC
 25                  B   ND      LPV       L   APTIAR  T   B   L      TR
                     0   LE      UBU       U   N1NACF  X   A   A      NF
                     2   11      122       1   221111  1   N   A      11
                                 //            / ///               1   4
                                                             1      /
        AGGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT
    601 --------------+-------------+-------------+-------------+-------------+-------------+ 660
 30     TCCCCCCCCTTCTGGGAGAGGCACAGAGTCGACCTCGAGGTCCTATCACCGTGGACCTGTA

          G  G  K  T  L  S  V  S  Q  L  E  L  Q  D  S  G  T  W  T  C -

 35           N                        M                   NM  A
              NS                       B                   HA  L
              LP                       0                   EE  U
              AH                       2                   11  1
 40           31
              /
        GCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAATAGACATCGTGGTGCTAGCTT
    661 --------------+-------------+-------------+-------------+-------------+-------------+ 720
        CGTGACAGAACGTCTTGGTCTTCTTCCACCTCAAGTTTTATCTGTAGCACCACGATCGAA
 45
          T  V  L  Q  N  Q  K  K  V  E  F  K  I  D  I  V  V  L  A  F -

 50


 55
```

```
                    HS          M  M
                    AT          N  N
                    EU          L  L
  5                 31          1  1
                    /
      TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAGTTCTCCTTCC
  721 ----------+----------+----------+----------+----------+----------+ 780
      AGGTCTTCCGGAGGTCGTATCAGATATTCTTTCTCCCCCTTGTCCACCTCAAGAGGAAGG
 10
       Q  K  A  S  S  I  V  Y  K  K  E  G  E  Q  V  E  F  S  F  P -

                                A                    A           M
                                L                    L           N
 15                             U                    U           L
                                1                    1           1
      CACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGGCAGGCGGAGA
 781 ----------+----------+----------+----------+----------+----------+ 840
      GTGAGCGGAAATGTCAACTTTTCGACTGCCCGTCACCGCTCGACACCACCGTCCGCCTCT
 20
        L  A  F  T  V  E  K  L  T  G  S  G  E  L  W  W  Q  A  E  R -

                           P  S
                  H    W  FW A                              W
                  P    N  LN U                              B
 25               H    L  ML 3                              0
                  1    1  11 A                              2
      GGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAAGTGTCTGTAA
 841 ----------+----------+----------+----------+----------+----------+ 900
      CCCGAAGGAGGAGGTTCAGAACCTAGTGGAAACTGGACTTCTTGTTCCTTCACAGACATT
 30
       A  S  S  S  K  S  W  I  T  F  D  L  K  N  K  E  V  S  V  K -

                  B         BS  PS
                  SM        SCADNPAD  A               A  H
 35               TA        TRVRLUUD  L               L  P
                  EE        NFAAAW9E  U               U  H
                  23        11224161  1               1  1
                  /         / /  //
      AACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTCCACCTCACCC
 40 901 ----------+----------+----------+----------+----------+----------+ 960
      TTGCCCAATGGGTCCTGGGATTCGAGGTCTACCCGTTCTTCGAGGGCGAGGTGGAGTGGG
       R  V  T  Q  D  P  K  L  Q  M  G  K  K  L  P  L  H  L  T  L -

                     BS
 45               M  SC HS       D        M  H          BSS
                  N  TR AT       D        N  P          SCAHM
                  L  NF EU       E        L  H          TRUAN
                  1  11 31       1        1  1          NF9EL
                     /  /                               11631
 50                  / /                                / /
      TGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCCCTTGAAGCGA
 961 ----------+----------+----------+----------+----------+----------+ 1020
      ACGGGGTCCGGAACGGAGTCATACGACCGAGACCTTTGGAGTGGGACCGGGAACTTCGCT
 55
       P  Q  A  L  P  Q  Y  A  G  S  G  N  L  T  L  A  L  E  A  K -
```

```
                    S           SA      BHF BS                        H
                  ANA         HNCP    SGNMAANXA                RSD I A
                  VLU         PCRA    PIUNMULHV                SCD N L
                  AA9         AIFL    1ADLH3AOA                AAE D U
                  236         2111    21211A421                111 3 1
                  //          //      / / / /                      /
     TCAAGGTTCTGCCCACATGGTCCACCCCGGTGCACGCGGATCCCGAGCGGTGAGTACTAAG
1261 -------------+------------+------------+------------+----------+ 1320
     AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCGCCTAGGGCTCCCACTCATGATTC

        K   V   L   P   T   W   S   T   P   V   H   A   D   P   E

                    E           BS              SS      F           BS      F
                H   CHH     F   SC          HHNCF   N           BSC     N
                P   OHA     O   TR          PGCRA   U           BTR     U
                H   4AE     K   NF          AAIFN   4           VNF     4
                1   712     1   11          21111   H           111     H
                /           /               //                  //
     CTTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGCAAGGC
1321 -------------+------------+------------+------------+----------+ 1380
     GAAGTCGCGAGGACGGACCTGCGTAGGGCCGATACGTCGGGGTCAGGTCCCGTCGTTCCG

                    S               S
                DBHVHNA         HVNCN           V           VNDV
                RBABPLU         PNCR_           N           N_DE
                AVEDHA9         ALIFA           L           LAED
                2132146         21114           1           1312
                // //           //
     AGGCCCCGTCTGCCTCTTCACCCGGAGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGG
1381 -------------+------------+------------+------------+----------+ 1440
     TCCGGGGCAGACGGAGAAGTGGGCCTCGGAGACGGGCGGGGTGAGTACGAGTCCCTCTCC

                    BS      P                           B       BS  S
                    SC      F               V   B   N   S       SCDHA
                    TR      L               A   A   L   P       TRRAU
                    NF      M               E   N   A   1       NFAE9
                    11      1               1   1   4   2       11236
                    /                                           / /
     GTCTTCTGGCTTTTTCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCC
1441 -------------+------------+------------+------------+----------+ 1500
     CAGAAGACCGAAAAAGGGTCCGAGACCCGTCCGTGTCCGATCCACGGGGATTGGGTCCGG

                    B                       B           B       S       PS
                    S                       DBS         S   M   HNC     ADNPA
                    P                       DAP         P   N   PCR     VRLUU
                    M                       EN1         M   L   AIF     AAAM9
                    1                       122         1   1   211     22416
                                            /                   /       / //
     CTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACC
1501 -------------+------------+------------+------------+----------+ 1560
     GACGTGTGTTTCCCCGTCCACGACCCGAGTCTGGACGGTTCTCGGTATAGGCCCTCCTGG
```

39

```
          D                H                D   A   M
          D                A                D   L   N
          E                E                E   U   L
          1                3                1   1   1
       CTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGACACC
5
1561   -----------+----------+----------+----------+----------+----------+   1620
       GACGGGGACTGGATTCGGGTGGGGTTTCCGGTTTGAGAGGTGAGGGAGTCGAGCCTGTGG


10        L  P  L  T  *  A  H  P  K  G  Q  T  L  H  S  L  S  S  D  T  -
          C  P  *  P  K  P  T  P  K  A  K  L  S  T  P  S  A  R  T  P  -
          A  P  D  L  S  P  P  Q  R  P  N  S  P  L  P  Q  L  G  H  L  -


          H
          I        M   MM                        DF                    S
15        N        N   AB                        DO                    F
          F        L   EO                        EK                    A
          1        1   32                        11                    N
                                                                       1
       TTCTCTCCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTCAGGGAGTGCATCCGCCCC
20
1621   -----------+----------+----------+----------+----------+----------+   1680
       AAGAGAGGAGGGTCTAAGGTCATTGAGGGTTAGAAGAGAGAGTCCCTCACGTAGGCGGGG

                                                        G  S  A  S  A  P  -
25


                                   E
                                   C
                           M       O
                           N       R
30                         L       1
                           1
       AACCCTTTTTCCCCCTCGTCTCCTGTGAGAATTCC....
1681   -----------+----------+----------+----   1714
       TTGGGAAAAGGGGGAGCAGAGGACACTCTTAAGG....

35        T  L  F  P  L  V  S  C  E  N  S  ....


40


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Table 4

```
        F N                S               B
        N S        B       M       H   DHA         B
        U P        B       N       G   RAU         S
        4 B        B       N       G   AE9         T
        H 2        V       L       A   236         X
                   1       1       1   /           1
    GCCTGTTTGAGAAGCAGCGGGCAAGAAAGACGCAAGCCCAGAGGCCCTGCCCATTTCTGTG
  1 -------------+-------------+-------------+-------------+----------+ 60
    CGGACAAACTCTTCGTCGCCCGTTCTTTCTGCGTTCGGGTCTCCGGGACGGTAAAGACAC


        B    PS                    S                          S
       DBS  ADNPA      D         DHNA            M   HM       HNC
       DAP  VRLUU      D         RALU            N   AN       PCR
       EN1  AAAM9      D         RALU            L   AN       PCR
       122  22416      E         AEA9            L   EL       AIF
            /  / //    1         2346            1   31       211
                        /         /               /           /
    GGCTCAGGTCCCTACTGGCTCAGGCCCCTGCCTCCCTCGGCAAGGCCACAATGAACCGGG
 61 -------------+-------------+-------------+-------------+----------+ 120
    CCGAGTCCAGGGATGACCGAGTCCGGGGACGGAGGGAGCCGTTCCGGTGTTACTTGGCCC

                                                        M   N   R   G -

        H                   B        F                  F   M   D
        I                   B        N                  N   M   D
        N                   B        U                  U   N   D
        F                   V        4        HH        4   L   E
        1                   1        H        HA        H   1   1
                                              AE
                                              12
    GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
121 -------------+-------------+-------------+-------------+----------+ 180
    CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG

        V   P   F   R   H   L   L   L   V   L   Q   L   A   L   L   P   A   A   T   Q -

        B        E        E                          R   A
        B        C        C                          S   L
        V        O        O                          A   U
        1        K        K                          1   1
    AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
181 -------------+-------------+-------------+-------------+----------+ 240
    TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA

        G   K   K   V   V   L   G   K   K   G   D   T   V   E   L   T   C   T   A   S -
```

41

```
                                                              H
                  M   M                                       I
                  B   B                                       N
                  O   O                                       F
                  2   2                                       1
    CCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAA
241 ------------+---------+---------+---------+---------+---------+ 300
    GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT

     Q  K  K  S  I  Q  F  H  W  K  N  S  N  Q  I  K  I  L  G  N -

              B                S              S     F     H
            NBS      F        AA        A    SAU   FN   HI
            LAP      O        VU        L    AU    NU   HN
            AN1      K        A9        U    3     DA   FA
            422      1        26        1    A     2 1  1 1
                     /        /
    ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
301 ------------+---------+---------+---------+---------+---------+ 360
    TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT

     Q  G  S  F  L  T  K  G  P  S  K  L  N  D  R  A  D  S  R  R -

              S                S        H     A           H
            MANAS             BA        I     A          ID
            BVLUT             CU        N     F          ND
            OAA9Y             L3        F     L          FE
            22461            1A         1     2          1 1
                /            /
    GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
361 ------------+---------+---------+---------+---------+---------+ 420
    CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA

     S  L  W  D  Q  G  N  F  P  L  I  I  K  N  L  K  I  E  D  S -

                              S
            M       M       AMAM                       M
            B       N       VNUN                       A
            O       L       AL9L                       E
            2       1       2161                       1
                             //
    CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
421 ------------+---------+---------+---------+---------+---------+ 480
    GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC

     D  T  Y  I  C  E  V  E  D  Q  K  E  E  V  Q  L  L  V  F  G -
```

```
5                                                 B                          S
                                                  S                          T
                                                  P                          Y
                                                  M                          1
                                                  1
      GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
10  481 --------+--------+--------+--------+--------+--------+ 540
      CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC
        L  T  A  N  S  D  T  H  L  L  Q  G  Q  S  L  T  L  T  L  E  -

          B   BS
          BS  SC                    D          M           H
15        AP  TR                    D          N           I    S
          N1  NF                    E          N           N    T
          22  11                    1          L           F    Y
                                               1           1    1
          /   /
      AGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC
20  541 --------+--------+--------+--------+--------+--------+ 600
      TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTTGTATG
        S  P  P  G  S  S  P  S  V  Q  C  R  S  P  R  G  K  N  I  Q  -

                              N           BBH S   B           BS
25            M    MD         ASP    A    BSSGSC  S   B   N   SC
              B    ND         LPV    L    APTIAR  T   A   L   TR
              0    LE         UBU    U    N1NACF  X   N   A   NF
              2    11         122    1    221111  1   1   4   11
                                    //         / ///           /
30    AGGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT
    601 --------+--------+--------+--------+--------+--------+ 660
      TCCCCCCCCTTCTGGGAGAGGCACAGAGTCGACCTCGAGGTCCTATCACCGTGGACCTGTA
        G  G  K  T  L  S  V  S  Q  L  E  L  Q  D  S  G  T  W  T  C  -

35    N                               M                       NM   A
      NS                              B                       HA   L
      LP                              0                       EE   U
      AH                              2                       11   1
      31
40    /
      GCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAATAGACATCGTGGTGCTAGCTT
    661 --------+--------+--------+--------+--------+--------+ 720
      CGTGACAGAACGTCTTGGTCTTCTTCCACCTCAAGTTTTATCTGTAGCACCACGATCGAA
        T  V  L  Q  N  Q  K  K  V  E  F  K  I  D  I  V  V  L  A  F  -
45
              HS          M    M
              AT          N    N
              EU          L    L
              31          1    1
50            /
      TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGACGGGGGAACAGGTGGAGTTCTCCTTCC
    721 --------+--------+--------+--------+--------+--------+ 780
      AGGTCTTCCGGAGGTCGTATCAGATATTCTTTCTCCCCCTTGTCCACCTCAAGAGGAAGG
55       Q  K  A  S  S  I  V  Y  K  K  E  G  E  Q  V  E  F  S  F  P  -
```

```
                              A                    A         M
                              L                    L         N
                              U                    U         L
                              1                    1         1

       CACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGGCAGGCGGAGA
781    ----------+---------+---------+---------+---------+---------+    840
       GTGAGCGGAAATGTCAACTTTTCGACTGCCCGTCACCGCTCGACACCACCGTCCGCCTCT
        L  A  F  T  V  E  K  L  T  G  S  G  E  L  W  W  Q  A  E  R  -

                            P  S
             H      M  FM   A                              W
             P      N  LN   U                              B
             H      L  ML   3                              O
             1      1  11   A                              2

       GGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAAGTGTCTGTAA
841    ----------+---------+---------+---------+---------+---------+    900
       CCCGAAGGAGGAGGTTCAGAACCTAGTGGAAACTGGACTTCTTGTTCCTTCACAGACATT
         A  S  S  S  K  S  W  I  T  F  D  L  K  N  K  E  V  S  V  K  -

             B         BS   PS
             SM        SCADNPAD    A                    A  H
             TA        TRVRLUUD    L                    L  P
             EE        NFAAAM9E    U                    U  H
             23        11224161    1                    1  1
             /          / / //
       AACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTCCACCTCACCC
901    ----------+---------+---------+---------+---------+---------+    960
       TTGCCCAATGGGTCCTGGGATTCGAGGTCTACCCGTTCTTCGAGGGCGAGGTGGAGTGGG
         R  V  T  Q  D  P  K  L  Q  M  G  K  K  L  P  L  H  L  T  L  -

                BS                                       BSS
             M  SC HS        D         M  H              SCAHM
             N  TR AT        D         N  P              TRUAN
             L  NF EU        E         L  H              NF9EL
             1  11 31        1         1  1              11631
                /  /                                       /   /
       TGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCCCTTGAAGCGA
961    ----------+---------+---------+---------+---------+---------+    1020
       ACGGGGTCCGGAACGGAGTCATACGACCGAGACCTTTGGAGTGGGACCGGGAACTTCGCT
         P  Q  A  L  P  Q  Y  A  G  S  G  N  L  T  L  A  L  E  A  K  -

                          S         BS
                          F         SC                H  D     A
                          A         TR                P  D     L
                          N         NF                H  E     U
                          1         11                1  1     1
                                     /
       AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGA
1021   ----------+---------+---------+---------+---------+---------+    1080
       TTTGTCCTTTCAACGTAGTCCTTCACTTGGACCACCACTACTCTCGGTGAGTCGAGGTCT

         T  G  K  L  H  Q  E  V  N  L  V  V  M  R  A  T  Q  L  Q  K  -
```

44

```
                              PS         S
        M                     ADNNPA     DF  AM     DE  A
        N                     VRLLUU     DA  LN     DS  L
        L                     AAAAV9     EN  UL     EP  U
        1                     224416     11  11     11  1
                              /////      /   /      /
        AAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAAAC
1081    ------------+---------+---------+---------+---------+---------+ 1140
        TTTTAAACTGGACACTCCACACCCCTGGGTGGAGGGGGATTCGACTACGACTCGAACTTTG
          N  L  T  C  E  V  W  G  P  T  S  P  K  L  M  L  S  L  K  L  -

        M                          T         H         M       DV
        N                          A         P         N       DS
        L                          Q         A         L       ET
        1                          1         2         1       12
                                                               /
        TGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGCCGGTGTGGGTGCTGAACCCTG
1141    ------------+---------+---------+---------+---------+---------+ 1200
        ACCTCTTGTTCCTCCGTTTCCAGAGCTTCGCCCTCTTCGGCCACACCCACGACTTGGGAC
          E  N  K  E  A  K  V  S  K  R  E  K  P  V  W  V  L  N  P  E  -

                            H              PS        H
                 F   D   M  I  A          ADPA      I
                 O   D   A  N  V          VRUU      N
                 K   E   E  F  A          AAV9      F
                 1   1   3  1  1          2216      1
                                          ///
        AGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTGGAATCCAACA
1201    ------------+---------+---------+---------+---------+---------+ 1260
        TCCGCCCCTACACCGTCACAGACGACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT
          A  G  M  W  Q  C  L  L  S  D  S  G  Q  V  L  L  E  S  N  I  -

                         S        SA    BHF BS                    H
                 ANA      HNCP   SGNVAANXA              RSD I A
                 VLU      PCRA   PIUNMULHV              SCD N L
                 AA9      AIFL   1ADLH3AOA              AAE D U
                 236      2111   21211A421             111 3 1
                 //       //     / / / /               /
        TCAAGGTTCTGCCCACATGGTCCACCCCGGTGCACGCGGATCCCGAGGGTGAGTACTAAG
1261    ------------+---------+---------+---------+---------+---------+ 1320
        AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCGCCTAGGGCTCCCACTCATGATTC
          K  V  L  P  T  W  S  T  P  V  H  A  D  P  E

                   E      BS             SS       F          BS   F
        H          CHH  F SC          HHNCF      N          BSC  N
        P          OHA  O TR          PGCRA      U          BTR  U
        H          4AE  K NF          AAIFN      4          VNF  4
        1          712  1 11          21111      H          111  H
        /                /            //                     //
        CTTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGCAAGGC
1321    ------------+---------+---------+---------+---------+---------+ 1380
        GAAGTCGCGAGGACGGACCTGCGTAGGGCCGATACGTCGGGGTCAGGTCCCGTCGTTCCG
```

45

```
              S                        S
          DBHI/HNA                  HMNCN            M           MNDV
          RBABPLU                   PNCRL            N           N_DS
          AVEDHA9                   ALIFA            L           LAEG
          2132146                   21114            1           1312
           //  //                    //
          AGGCCCCGTCTGCCTCTTCACCCGGAGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGG
    1381  --------+---------+---------+---------+---------+---------+  1440
          TCCGGGGCAGACGGAGAAGTGGGCCTCGGAGACGGGCGGGGTGAGTACGAGTCCCTCTCC

                      BS   P                    M   B   N   B       BS  S
                      SC   F                    A   A   L   S       SCDHA
                      TR   L                    E   L   A   P       TRRAU
                      NF   M                        N       1       NFAE9
                      11   1                    1   1   4   2       11236
                                                                     /   /
          GTCTTCTGGCTTTTTCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCC
    1441  --------+---------+---------+---------+---------+---------+  1500
          CAGAAGACCGAAAAAGGGTCCGAGACCCGTCCGTGTCCGATCCACGGGGATTGGGTCCGG

                      B                B            B       M       S        PS
                      S                DBS          S       M       HNC      ADNPA
                      P                DAP          P       N       PCR      VRLUU
                      M                EN1          M       L       AIF      AAAV9
                      1                122          1       1       211      22416
                                        /                            /       / //
          CTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACC
    1501  --------+---------+---------+---------+---------+---------+  1560
          GACGTGTGTTTCCCCGTCCACGACCCGAGTCTGGACGGTTCTCGGTATAGGCCCTCCTGG

                      D                H                    D       A       M
                      D                A                    D       L       N
                      E                E                    E       U       L
                      1                3                    1       1       1
          CTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGACACC
    1561  --------+---------+---------+---------+---------+---------+  1620
          GACGGGGACTGGATTCGGGTGGGGTTTCCGGTTTGAGAGGTGAGGGAGTCGAGCCTGTGG

                      H
                      I    M    MM                      BP      DE      F
                      N    N    AB                      BS      DS      AN
                      F    L    EO                      VT      EP      LU
                      1    1    32                      11      11      U4
                                  /                      /       /      1H
          TTCTCTCCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGTGATTGCTGAGCTGC
    1621  --------+---------+---------+---------+---------+---------+  1680
          AAGAGAGGAGGGTCTAAGGTCATTGAGGGTTAGAAGAGAGACGTCACTAACGACTCGACG

                                                      V   I   A   E   L   P  -
```

```
                                              F
    M  H     M                        M       N
    B  G     N                        B       U
    D  A     L                        D       D
    2  1     1                        2       2
      CTCCCAAAGTGAGCGTCTTCGTCCCACCCCGCGACGGCTTCTTCGGCAACCCCCGCAAGT
1681  ------------+---------+---------+---------+---------+----------+  1740
      GAGGGTTTCACTCGCCAGAAGCAGGGTGGGGCGCTGCCGAAGAAGCCGTTGGGGGCGTTCA

       P  K  V  S  V  F  V  P  P  R  D  G  F  F  G  N  P  R  K  S -

                   BS              S     H              B S F
             A     SC H            HNC   I     B        SMC N
             L     TR A            PCR   N     B        TNR U
             U     NF E            AIF   F     V        NLF 4
             1     11 3            211   1     1        111 H
                    /              //                   ///
      CCAAGCTCATCTGCCAGGCCACGGGTTTCAGTCCCCGGCAGATTCAGGTGTCCTGGCTGC
1741  ------------+---------+---------+---------+---------+----------+  1800
      GGTTCGAGTAGACGGTCCGGTGCCCAAAGTCAGGGGCCGTCTAAGTCCACAGGACCGACG

       K  L  I  C  Q  A  T  G  F  S  P  R  Q  I  Q  V  S  W  L  R -

        F  B                        S    BS                      H
        NH S          H  H    AM     AA   SCM       D     H      I
        UH P          P  G    HA     VU   TRN       D     A      N
        DA M          H  A    AE     A9   NFL       E     E      F
        21 1          1  1    23     26   111       1     3      1
         /                           /     /
      GCGAGGGGAAGCAGGTGGGGTCTGGCGTCACCACGGACCAGGTGCAGGCTGAGGCCAAAG
1801  ------------+---------+---------+---------+---------+----------+  1860
      CGCTCCCCTTCGTCCACCCCAGACCGCAGTGGTGCCTGGTCCACGTCCGACTCCGGTTTC

       E  G  K  Q  V  G  S  G  V  T  T  D  Q  V  Q  A  E  A  K  E -

          SS     B              B
          AAHNABS               SM          H
          UUALPAP               TA          P
          99EAAN1               EE          H
          6634122               23          1
            /  //                 /
      AGTCTGGGCCCACGACCTACAAGGTGACCAGCACACTGACCATCAAAGAG....
1861  ------------+---------+---------+---------+----------+  1910
      TCAGACCCGGGTGCTGGATGTTCCACTGGTCGTGTGACTGGTAGTTTCTC....

       S  G  P  T  T  Y  K  V  T  S  T  L  T  I  K  E ....
```

Table 5

```
                    F N                                         S                   B
                    N S                    B       M       H    DHA                 S
                    U P                    B       M       G    RAU                 T
                    4 B                    V       L       A    AE9                 X
                    H 2                    1       1       1    236                 1
                                                                  /
          GCCTGTTTGAGAAGCAGCGGGCAAGAAAGACGCAAGCCCAGAGGCCCTGCCATTTCTGTG
        1 ---------+---------+---------+---------+---------+---------+ 60
          CGGACAAACTCTTCGTCGCCCGTTCTTTCTGCGTTCGGGTCTCCGGGACGGTAAAGACAC


               B    PS                        S                               S
             DBS  ADNPA       D           DHNA            M      HM         HNC
             DAP  VRLUU       D           RALU            N      AN         PCR
             EN1  AAAM9       E           AEA9            L      EL         AIF
             122  22416       1           2346           1      31         211
               /  / //                       /                  /             /
          GGCTCAGGTCCCTACTGGCTCAGGCCCCTGCCTCCCTCGGCAAGGCCACAATGAACCGGG
       61 ---------+---------+---------+---------+---------+---------+ 120
          CCGAGTCCAGGGATGACCGAGTCCGGGGACGGAGGGAGCCGTTCCGGTGTTACTTGGCCC


                                                                  M   N   R   G -

             H                      B                F              F
             I                      B                N            HH  N  M   D
             N                      B                U            HA  U  N   D
             F                      V                4            AE  4  L   E
             1                      1                H            12  H  1   1
          GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
      121 ---------+---------+---------+---------+---------+---------+ 180
          CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


             V   P   F   R   H   L   L   L   V   L   Q   L   A   L   L   P   A   A   T   Q -

                 B       E   E                                              R     A
                 B       C   C                                              S     L
                 V       O   O                                              A     U
                 1       K   K                                              1     1
          AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
      181 ---------+---------+---------+---------+---------+---------+ 240
          TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA


             G   K   K   V   V   L   G   K   K   G   D   T   V   E   L   T   C   T   A   S -

                             M   M                                   H
                             B   B                                   I
                             0   0                                   N
                             2   2                                   F
                                                                     1
          CCCAGAAGAAGAGCATACAATTCCACTGGAAAAAACTCCAACCAGATAAAGATTCTGGGAA
      241 ---------+---------+---------+---------+---------+---------+ 300
          GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT


             Q   K   K   S   I   Q   F   H   W   K   N   S   N   Q   I   K   I   L   G   N -
```

48

```
                     B                    S                         S     F     H
                     NBS         F        AA           A            A     N     H     I
                     LAP         0        VU           L            U     H     H     N
                     AN1         K        A9           U            3     U     H     F
                     422         1        26           1            A     D     A     1
                                 /                 /                      2     1
     ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
301  --------------+----------+----------+----------+----------+----------+  360
     TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT

        Q   G   S   F   L   T   K   G   P   S   K   L   N   D   R   A   D   S   R   R -


                     S                              S          H      A           H
              MANAS                                 BA         I      F           I     D
              BVLUT                                 CU         N      L           N     D
              0AA9Y                                 L3         F      L           F     E
              22461                                 1A         1      2           1     1
                                  /                       /
     GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
361  --------------+----------+----------+----------+----------+----------+  420
     CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA

        S   L   W   D   Q   G   N   F   P   L   I   I   K   N   L   K   I   E   D   S -


                                                S
                     M          M               AMAM                     M
                     B          N               VNUN                     A
                     0          L               AL9L                     E
                     2          1               2161                     1
                                                 //
     CAGATACTTACATCTGTGAAGTGGAGGACCAGAACGAGGAGGTGCAATTGCTAGTGTTCG
421  --------------+----------+----------+----------+----------+----------+  480
     GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC

        D   T   Y   I   C   E   V   E   D   Q   K   E   E   V   Q   L   L   V   F   G -


                                           B                          S
                                           S                          T
                                           P                          Y
                                           M                          1
                                           1
     GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
481  --------------+----------+----------+----------+----------+----------+  540
     CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC

        L   T   A   N   S   D   T   H   L   L   Q   G   Q   S   L   T   L   T   L   E -
```

```
              B   BS                                        H
              BS  SC              D                  M      I        S
              AP  TR              D                  N      N        T
              N1  NF              E                  L      F        Y
              22  11              1                  1      1        1
               /   /
         AGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC
     541 --------+---------+---------+---------+---------+---------+ 600
         TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTTGTATG
           S  P  P  G  S  S  P  S  V  Q  C  R  S  P  R  G  K  N  I  Q -


                                 N           BBH S    B                BS
                   M   MD        ASP    A     BSSGSC   S     B    N     SC
                   B   ND        LPV    L     APTIAR   T     A    L     TR
                   0   LE        UBU    U     N1NACF   X     N    A     NF
                   2   11        122    1     221111   1     1    4     11
                                  //            /  ///                  /
         AGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT
     601 --------+---------+---------+---------+---------+---------+ 660
         TCCCCCCCTTCTGGGAGAGGCACAGAGTCGACCTCGAGGTCCTATCACCGTGGACCTGTA
           G  G  K  T  L  S  V  S  Q  L  E  L  Q  D  S  G  T  W  T  C -


           N
           NS                              M                    NM   A
           LP                              B                    HA   L
           AH                              0                    EE   U
           31                              2                    11   1
            /
         GCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAAATAGACATCGTGGTGCTAGCTT
     661 --------+---------+---------+---------+---------+---------+ 720
         CGTGACAGAACGTCTTGGTCTTCTTCCACCTCAAGTTTTATCTGTAGCACCACGATCGAA
           T  V  L  Q  N  Q  K  K  V  E  F  K  I  D  I  V  V  L  A  F -


               HS         M   M
               AT         N   N
               EU         L   L
               31         1   1
                /
         TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAGTTCTCCTTCC
     721 --------+---------+---------+---------+---------+---------+ 780
         AGGTCTTCCGGAGGTCGTATCAGATATTCTTTCTCCCCCTTGTCCACCTCAAGAGGAAGG
           Q  K  A  S  S  I  V  Y  K  K  E  G  E  Q  V  E  F  S  F  P -


                          A                    A         M
                          L                    L         N
                          U                    U         L
                          1                    1         1
         CACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGGCAGGCGGAGA
     781 --------+---------+---------+---------+---------+---------+ 840
         GTGAGCGGAAATGTCAACTTTTCGACTGCCCGTCACCGCTCGACACCACCGTCCGCCTCT
           L  A  F  T  V  E  K  L  T  G  S  G  E  L  W  W  Q  A  E  R -
```

```
                    P  S
       H      M  FN A                                        M
       P      N  LN U                                        B
       H      L  ML 3                                        0
       1      1  11 A                                        2
       GGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAAGTGTCTGTAA
 841   --------+---------+---------+---------+---------+---------+   900
       CCCGAAGGAGGAGGTTCAGAACCTAGTGGAAACTGGACTTCTTGTTCCTTCACAGACATT

        A  S  S  S  K  S  W  I  T  F  D  L  K  N  K  E  V  S  V  K -

       B            BS   PS
       SM           SCADNPAD   A                A  H
       TA           TRVRLUUD   L                L  P
       EE           NFAAAM9E   U                U  H
       23           11224161   1                1  1
           ▼         / / //
       AACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTCCACCTCACCC
 901   --------+---------+---------+---------+---------+---------+   960
       TTGCCCAATGGGTCCTGGGATTCGAGGTCTACCCGTTCTTCGAGGGGCGAGGTGGAGTGGG

        R  V  T  Q  D  P  K  L  Q  M  G  K  K  L  P  L  H  L  T  L -

             BS                                    BSS
       M     SC HS         D         M  H          SCAHM
       N     TR AT         D         N  P          TRUAN
       L     NF EU         E         L  H          NF9EL
       1     11 31         1         1  1          11631
              /  /                                  /  /
       TGCCCCAGGCCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCCCCTTGAAGCGA
 961   --------+---------+---------+---------+---------+---------+   1020
       ACGGGGTCCGGGAACGGAGTCATACGACCGAGACCTTTGGAGTGGGACCGGGAACTTCGCT

        P  Q  A  L  P  Q  Y  A  G  S  G  N  L  T  L  A  L  E  A  K -

                    S         BS
                    F         SC                 H  D  A
                    A         TR                 P  D  L
                    N         NF                 H  E  U
                    1         11                 1  1  1
                              /
       AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGA
1021   --------+---------+---------+---------+---------+---------+   1080
       TTTGTCCTTTCAACGTAGTCCTTCACTTGGACCACCACTACTCTCGGTGAGTCGAGGTCT

        T  G  K  L  H  Q  E  V  N  L  V  V  M  R  A  T  Q  L  Q  K -
```

51

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                              PS              S
           M              ADNNPA          DF    AM        DE   A
           N              VRLLUU          DA    LN        DS   L
   5       L              AAAAM9          EN    UL        EP   U
           1              224416          11    11        11   1
                          /////           /     /         /
        AAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAAAC
  1081  --------+---------+---------+---------+---------+---------+  1140
   10   TTTTAAACTGGACACTCCACACCCCTGGGTGGAGGGGATTCGACTACGACTCGAACTTTG

           N   L   T   C   E   V   W   G   P   T   S   P   K   L   M   L   S   L   K   L  -

           M                       T                   H               M           DM
   15      N                       A                   P               N           DS
           L                       Q                   A               L           ET
           1                       1                   2               1           12
                                                                                    /
        TGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGCCGGTGTGGGTGCTGAACCCTG
   20 1141 --------+---------+---------+---------+---------+---------+  1200
        ACCTCTTGTTCCTCCGTTTCCAGAGCTTCGCCCTCTTCGGCCACACCCACGACTTGGGAC

           E   N   K   E   A   K   V   S   K   R   E   K   P   V   W   V   L   N   P   E  -

                                   H                   PS          H
   25                      F   D   M   I   A       ADPA            I
                           O   D   A   N   V       VRUU            N
                           K   E   E   F   A       AAM9            F
                           1   1   3   1   1       2216            1
                                                    ///
   30   AGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGCACAGGTCCTGCTGGAATCCAACA
   1201 --------+---------+---------+---------+---------+---------+  1260
        TCCGCCCCTACACCGTCACAGACGACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT

           A   G   M   W   Q   C   L   L   S   D   S   G   Q   V   L   L   E   S   N   I  -
   35
                           S           SA      BHF BS                          B
                         ANA         HNCP    SGNMAANXA                        SH
                         VLU         PCRA    PIUNMULHV                        PP
   40                    AA9         AIFL    1ADLH3AOA                        1H
                         236         2111    21211A421                        21
                          //          //      / / /
        TCAAGGTTCTGCCCACATGGTCCACCCCGGTGCACGCGGATCCCGAGGGTGAGTGTGCCC
   1261 --------+---------+---------+---------+---------+---------+  1320
   45   AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCGCCTAGGGCTCCCACTCACACGGG

           K   V   L   P   T   W   S   T   P   V   H   A   D   P   E
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                     BS  S
     MF              SC  F      DHNA      HNC          A  M  M
     AO              TR  A      RALU      PCR          F  A  B
     EK              NF  N      AEA9      AIF          L  E  O
     11              11  1      2346      211          3  2  2
      /               /          /         /
     TAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGACACGTCCACCTCCATCT
1321 --------+---------+---------+---------+---------+---------+ 1380
     ATCTCATCGGACGTAGGTCCCTGTCCGGGGTCGGCCCACGACTGTGCAGGTGGAGGTAGA


                     BS      S
     M  D         M  SC  M ANA M                        M        S
     N  D         N  TR. B VLU B                        N        T
     L  E         L  NF  0 AA9 0                        L        Y
     1  1         1  11  2 246 2                        1        1
                      /       /
     CTTCCTCAGCACCTGAACTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCCCCCAAAACCCA
1381 --------+---------+---------+---------+---------+---------+ 1440
     GAAGGAGTCGTGGACTTGAGGACCCCCCTGGCAGTCAGAAGGAGAAGGGGGGGTTTTGGGT
      A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K  -


                 S           SS                  N
                 AN    M HMANNAC DM M             NS               M
                 UL    N PNVCLUR DS A             LP               A
                 3A    L ALAIA9F ET E             AH               E
                 A3    1 2121461 12 3             31               2
                        /  / //   /                /
     AGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCC
1441 --------+---------+---------+---------+---------+---------+ 1500
     TCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAGTGTACGCACCACCACCTGCACTCGG
      D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  -


     M         DM M                 RM M.
     N         DS B                 SA N
     L         ET 0                 AE L
     1         12 2                 12 1
               /
     ACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCA
1501 --------+---------+---------+---------+---------+---------+ 1560
     TGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCGCACCTCCACGTATTACGGT
      E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  -


               F FN                         S
        M      N NSS           R       M  R HNC HH
        N      U UPA           S       A  S PCR GP
        L      4 DBC           A       E  A AIF AH
        1      H 222           1       2  1 211 11
                  //                       /
     AGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCG
1561 --------+---------+---------+---------+---------+---------+ 1620
     TCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGCATGGCCCACCAGTCGCAGGAGTGGC
      T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  -
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                              BS
                              SC                           R
                    M         TR                           S
                    N         NF                           A
                    L         11                           1
                    1          /
         TCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC
    1621 --------+---------+---------+---------+---------+---------+ 1680
         AGGACGTGGTCCTGACCGACTTACCGTTCCTCATGTTCACGTTCCAGAGGTTGTTTCGGG

           L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L -

                                                    P S            S
                    M          T                    ADNNPMA        A
                    N          A                    VRLLUNU        U
                    L          Q                    AAAAML9        9
                    1          1                    2244116        6
                                                    //// /
         TCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGTGGGACCCGTGGGGTGCGAG
    1681 --------+---------+---------+---------+---------+---------+ 1740
         AGGGTCGGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCACCCTGGGCACCCCACGCTC

           P  A  P  I  E  K  T  I  S  K  A  K

                                     S                      N
                    H M      N     HHN  BSAH       D  M  M   S   R
                    A N      L     APA  GFUA       D  N  A   P   S
                    E L      A     EAE  LI9E       E  N  E   B   A
                    3 1      3     321  1163       1  1  3   2   1
                                     /
         GGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTGCCCTGAGAGTGACCGCTGTACCA
    1741 --------+---------+---------+---------+---------+---------+ 1800
         CCGGTGTACCTGTCTCCGGCCGAGCCGGGTGGGAGACGGGACTCTCACTGGCGACATGGT

                             F                              SS
                    M        N      A      B       R  F     AHNNCC
                    N        U      V      B       S  0     VPCCRR
                    L        4      A      V       A  K     AAIIFF
                    1        H      1      1       1  1     121111
                                                              ////
         ACCTCTGTCCTACAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGG
    1801 --------+---------+---------+---------+---------+---------+ 1860
         TGGAGACAGGATGTCCCGTCGGGGCTCTTGGTGTCCACATGTGGGACGGGGGTAGGGCCC

              G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  D -
```

54

```
                    BS              BS  B
S      A     F      SC              SC  S
M      L     O      TR              TR  P
A      U     K      NF              NF  M
1      1     1      11              11  1
/            /      /                   /
ATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCG
1861 --------+---------+---------+---------+---------+---------+ 1920
TACTCGACTGGTTCTTGGTCCAGTCGGACTGGACGGACCAGTTTCCGAAGATAGGGTCGC

     E   L   T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D -
                                     F
                                     N   H               B
                                     U   P               B
                                     4   A               V
                                     H   2               1
ACATCGCCGTGGAGTGGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTC
1921 --------+---------+---------+---------+---------+---------+ 1980
TGTAGCGGCACCTCACCCTCTCGTTACCCGTCGGCCTCTTGTTGATGTTCTGGTGCGGAG

      I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T   P  P -
          H                                                               B
      M   I   M           N               H           M   A               S
      N   N   B           L               P           N   L               P
      L   F   0           A               H           L   U               M
      1   1   2           4               1           1   1               1
CCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA
1981 --------+---------+---------+---------+---------+---------+ 2040
GGCACGACCTGAGGCTGCCGAGGAAGAAGGAGATGTCGTTCGAGTGGCACCTGTTCTCGT

      V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V   D   K   S  R -
      F                               S
      N M             M B X           N F   M                   N N
      U B             A B M           L A   N                   S L
      4 0             E V N           A N   L                   I A
      H 2             2 1 1           3 1   1                   1 3
                                      /
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT
2041 --------+---------+---------+---------+---------+---------+ 2100
CCACCGTCGTCCCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGA

      W   Q   Q   G   N   V   F   S   C   S   V   M   H   E   A   L   H   N   H Y -
                                      S
                  M   M   H N C                       C X H
                  B   N   P C R                       F M A
                  0   L   A I F                       R A E
                  2   1   2 1 1                       1 3 3
                          /                               /
ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCGACGGCCG
2101 --------+---------+---------+---------+---------+ 2150
TGTGCGTCTTCTCGGAGAGGGACAGAGGCCCATTTACTCACGCTGCCGGC

      T   Q   K   S   L   S   L   S   P   G   K   •
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Example 2: Preparation of the Fusion Proteins from Supernatants of COS Cells

COS cells grown in DME medium supplemented with 10% Calf Serum and gentamicin sulfate at 15 μg/ml were split into DME medium containing 10% NuSerum (Collaborative Research) and gentamicin to give 50% confluence the day before transfection. The next day, CsCl purified plasmid DNA was added to a final concentration of 0.1 to 2.0 μg/ml followed by DEAE Dextran to 400 μg/ml and chloroquine to 100 μM. After 4 hours at 37° C, the medium was aspirated and a 10% solution of dimethyl sulfoxide in phosphate buffered saline was added for 2 minutes, aspirated, and replaced with DME/10% Calf Serum. 8 to 24 hours later, the cells were trypsinized and split 1:2.

For radiolabeling, the medium was aspirated 40 to 48 hours after transfection, the cells washed once with phosphate buffered saline, and DME medium lacking cysteine or methionine was added. 30 minutes later, ^{35}S-labeled cysteine and methionine were added to final concentrations of 30-60 μci and 100-200 μci respectively, and the cells allowed to incorporate label for 8 to 24 more hours. The supernatants were recovered and examined by electrophoresis on 7.5% polyacrylamide gels following denaturation and reduction, or on 5% polyacrylamide following denaturation without reduction. The CD4Bγ1 protein gave the same molecular mass with or without reduction, while the CD4Eγ1 and CD4Hγ1 fusion proteins showed molecular masses without reduction of twice the mass observed with reduction, indicating that they formed dimer structures. The CD4 IgM fusion proteins formed large multimers beyond the resolution of the gel system without reduction, and monomers of the expected molecular mass with reduction.

Unlabeled proteins were prepared by allowing the cells to grow for 5 to 10 days post transfection in DME medium containing 5% NuSerum and gentamicin as above. The supernatants were harvested, centrifuged, and purified by batch adsorption to either protein A trisacryl, protein A agarose, goat anti-human IgG antibody agarose, rabbit anti-human IgM antibody agarose, or monoclonal anti-CD4 antibody agarose. Antibody agarose conjugates were prepared by coupling purified antibodies to cyanogen bromide activated agarose according to the manufacturer's recommendations, and using an antibody concentration of 1 mg/ml. Following batch adsorption by shaking overnight on a rotary table, the beads were harvested by pouring into a sintered glass funnel and washed a few times on the funnel with phosphate buffered saline containing 1% Nonidet P40 detergent. The beads were removed from the funnel and poured into a small disposable plastic column (Quik-Sep QS-Q column, Isolab), washed with at least 20 column volumes of phosphate buffered saline containing 1% Nonidet P40, with 5 volumes of 0.15 N NaCl, 1 mM EDTA (pH 8.0), and eluted by the addition of either 0.1 M acetic acid, 0.1 M acetic acid containing 0.1 M NaCl, or 0.25 M glycine-HCl buffer, pH 2.5.

Example 3: Blockage of Syncytium Formation by the Fusion Proteins

Purified or partially purified fusion proteins were added to HPB-ALL cells infected 12 hours previously with a vaccinia virus recombinant encoding HIV envelope protein. After incubation for 6-8 more hours, the cells were washed with phosphate buffered saline, fixed with formaldehyde, and photographed. All of the full-length CD4 immunoglobulin fusion proteins showed inhibition of syncytium formation at a concentration of 20 μg/ml with the exception of the 4Hγ1 protein, which was tested only at 5 μg/ml and showed partial inhibition of syncytium formation under the same conditions.

Example 4: Chromium Release Cytolysis Assay

The purified fusion proteins were examined for ability to fix complement in a chromium release assay using vaccinia virus infected cells as a model system. Namalwa (B cell) or HPB-ALL (T cell) lines were infected with vaccinia virus encoding HIV envelope protein, and 18 hours later were radiolabeled by incubation in 1 mci/ml sodium ^{51}chromate in phosphate buffered saline for 1 hour at 37°. The labeled cells were centrifuged to remove the unincorporated chromate, and incubated in microtiter wells with serial dilutions of the CD4 immunoglobulin fusion proteins and rabbit complement at a final concentration of 40%. After 1 hour at 37°, the cells were mixed well, centrifuged, and the supernatants counted in a gamma-ray counter. No specific release could be convincingly documented.

Example 5: Binding of the CD4Eγ1 Protein to Fc Receptors

Purified CD4Eγ1 fusion protein was tested for its ability to displace radiolabeled human IgG1 from human Fc receptors expressed on COS cells in culture. The IgG1 was radiolabeled with sodium [125]iodide

5 using 1 mci of iodide, 100 μg of IgG1, and two idobeads (Pierce). The labeled protein was separated from unincorporated counts by passage over a Sephadex G25 column equilibrated with phosphate buffered saline containing 0.5 mM EDTA and 5% nonfat milk. Serial dilutions of the CO4Eγ1 fusion protein or unlabeled IgG1 were prepared and mixed with a constant amount of radiolabeled IgG1 tracer. After incubation with COS cells bearing the FcRI and RcRII receptors at 4°C for at least 45 minutes in a volume

10 of 20 μl, 200 μl of a 3:2 mixture of dibutyl to dioctyl phthalates were added, and the cells separated from the unbound label by centrifugation in a microcentrifuge for 15 to 30 seconds. The tubes were cut with scissors, and the cell pellets counted in a gamma-ray counter. The affinity of the CD4Eγ1 protein for receptors was measured in parallel with the affinity of the authentic IgG1 protein, and was found to be the same, within experimental error.

15

Example 6: Stable Expression of the Fusion Construct pCD4Eγ1 in Baby Hamster Kidney Cells

Twenty-four hours before transfection, 0.5×10^6 baby hamster kidney cells (BHK; ATCC CCL10) were

20 seeded in a 25 cm² culture flask in Dulbecco's modified Eagle's medium (DMEN) containing 10% of fetal calf serum (FCS). The cells were cotransfected with a mixture of the plasmids pCD4Eγ1 (20 μg), pSV2dhfr (5 μg; Lee et al., Nature 294:228-232 (1981)) and pRMH140 (5 μg, Hudziak et al., Cell 31:137-146 (1982)) according to a modified calcium phosphate transfection technique as described in Zettlmeissl et al. (Behring Inst. Res. Comm. 82:26-34 (1988)). 72 h post-transfection, cells were split 1:3 to 1:4 (60 mm culture dishes)

25 and resistant colonies were selected in DMEM medium containing 10% FCS, 400 μg/ml G418 (Geneticin, Gibco) and 1 μM methotrexate (selection medium). The medium was changed twice a week. The resistant colonies (40-100/transfection) appeared 10-15 day post-transfection and were further propagated either as a mixture of clones (i.e., BHK-NK1) or as individually isolated clones. For the determination of the relative expression levels, clone mixtures or individual clones were grown to confluency in T25 culture flasks,

30 washed twice with protein-free DMEM medium, and incubated for 24 h with 5 ml protein-free DMEM medium. These media were collected and subjected to a human IgG specific ELISA in order to determine the relative expression levels of the CD4-IgG1 fusion protein CD4Eγ1. For further analysis an individual clone (BHK-UC3) was chosen due to its high relative expression levels.

35

Example 7: Detection of the CD4Eγ1 Protein in Culture Supernatants

For ^{35}S methionine labeling of cells, the clone BHK-UC3 and untransfected BHK cells (control) were grown to confluency in T25 culture flasks and subsequently incubated for two hours in HamF12 medium

40 without methionine. Labeling was achieved by incubating 24 h in 2.5 ml of the same medium containing 100 μCi ^{35}S methionine (1070 Ci/mmole, Amersham). For the preparation of cell lysates, the labeled cells were harvested in 1 ml of phosphate buffered saline, pH 7.2 (PBS) and lysed by repetitive freezing and thawing. Cleared lysates (after centrifugation 20000 rpm, 20 min) and culture supernatants were incubated with Protein A-Sepharose (Pharmacia) and the bound material was analyzed on a 10% SDS-Protein A-Sepharose

45 (Pharmacia) and the bound material was analyzed on a 10% SDS-gel according to Laemmli (Nature 227:680-685 (1970)), which was subsequently autoradiographed. A specific band of about 80 KDa can be detected only in the supernatant of clone BHK-UC3, which is absent in the lysate of clone BHK-UC3 and in the respective controls.

50

Example 8: Purification of the Protein CD4Eγ1 from Culture Supernatants

In order to demonstrate that the fusion protein coded by the plasmid pCD4Eγ1 can be obtained in high quantities, the clone BHK-UC3 was grown in 1750 cm² roller bottles in selection medium (500 ml). Confluent

55 monolayers were washed twice with protein-free DMEM medium (200 ml) and further incubated for 48 h with protein-free DMEM medium (500 ml). The conditioned culture supernatants (1-2 l) and respective supernatants from untransfected BHK cells were cleared by centrifugation (9000 rpm, 30 min) and microfiltered through a 0.45 μm membrane (Nalgene). After addition of 1% (v/v) of 1.9 M Tris-HCl buffer,

pH 8.6, the conditioned medium was absorbed to a Protein A-Sepharose column equilibrated with 50 mM Tris-HCl pH 8.6 buffer containing 150 mN NaCl (4°C). The loaded column was washed with 10 column volumes of equilibration buffer. Elution of the CD4-IgG1 fusion protein CD4Eγ1 was achieved with 0.1 M sodium citrate buffer, pH 3, followed by immediate neutralization of the column efflux to pH 8 by Tris-base.
5 The peak fractions were pooled, and the pool was analyzed on a Coomassie blue stained SDS-gel resulting in a band of the expected size (80 KDa), and which reacted with a polyclonal anti-human IgG heavy chain antibody and a mouse monoclonal anti-CD4 anti body (BMA040, Behringwerke) in Western Blots. The yields of purified fusion proteins obtained by the given procedure is 5-18 mg/24 h/l culture supernatant. The respective value for a BHK clone mixture (about 80 resistant clones; BHK-NK1) as described above was 2-3
10 mg/24 h/l.

Example 9: Physical and Biological Characterization of the CD4Eγ1 Fusion Protein

15 As proven by SDS-electrophoresis on 10-15% gradient gels (Phast-System, Pharmacia) under non-reductive conditions, the CD4Eγ1 fusion protein migrates at the position of a homodimer (about 160 KDa) like a non-reduced mouse monoclonal antibody. This result is supported by analytical equilibrium ultracentrifugation, where the fusion protein behaves as a homogeneous dimeric molecule of about 150 KDa. The absorbance coefficient of the protein was determined as A_{280} = 18 cm^2/mg using the quantitative protein
20 determination according to Bradford (Anal. Biochem. 72:248-254 (1976)).
   The CD4Eγ1-fusion protein shows specific complex formation with a solubilized βgal-gp120 fusion protein (pMB1790; Broker et al., Behring Inst. Res. Commun. 82:338-348 (1988)) expressed in E. coli. In this protein (110 KDa), a major part of the HIV gp120 protein (Val$_{49}$-Trp$_{645}$) is fused to β-galactosidase (amino acids 1-375). In a control experiment a 67-KDa β-gal-HIV 3'orf fusion protein (βgal1-375; 3'orf Pro14-
25 Asp123) showed no complex formation. En these experiments, the CD4Eγ1-protein was incubated with the respective fusion protein in molar rations of about 5:1. The complex was isolated by binding to Protein A-Sepharose and the Protein A-Sepharose bound proteins--together with relevant controls--were analyzed on 10-15% gradient SDS-gels (Phast-System, Pharmacia).
   The CD4Eγ1 fusion protein binds to the surface of HIV (HIV1/HTLV-IIIB) infected cultured T4-
30 lymphocytes as determined by direct immunofluorescence with fluorescein-isothiocyanate (FITC) labeled CD4Eγ1 protein. It blocks syncytia formation in cultured T4-lymphocytes upon HIV infection (0.25 TCID/cell) at a concentration of 10 μg/ml. Furthermore, HIV-infected cultured T4-lymphocytes (subclone of cell line H9) are selectively killed upon incubation with CD4Eγ1 in the presence or absence of complement. To a highly (>50%) HIV infected culture of T4-lymphocytes (10^6 cells/ml) 50, 10 or 1 μg/ml CD4Eγ1 fusion
35 protein was added in the presence or absence of guinea pig complement. Cells were observed for specific killing by the fusion protein, which is defined by the percentage of killed cells after 3 days in relation to viable cells in the culture at the beginning of the experiment corrected by the values for unspecific killing observed in control cultures, lacking the CD4Eγ1 fusion protein (Table 5, Experiment I). Surprisingly, addition of CD4Eγ1 protein to the infected T4 cells in the absence of complement resulted in similar
40 specific killing rates as in the presence of complement (Table 5, Experiment II). This result demonstrates a complement independent cytolytic effect of CD4Ey1 on HIV infected T-lymphocytes in culture.

Table 5

No. Experiment	Assay System	Specific Killing (%)
I	non-infected T4-cells + 50 μg/ml CD4Eγl + Compl.	0.7
	infected T4-cells + 50 μg/ml CD4Eγl + Compl.	35.1
	infected T4-cells + 10 μg/ml CD4Eγl + Compl.	25.1
	infected T4-cells + 1 μg/ml CD4Eγl + Compl.	25
II	infected T4-cells + 10 μg/ml CD4Eγl + Compl.	49.9
	infected T4-cells + 10 μg/ml CD4Eγl + Compl.	69.4

55 Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed with any wide range of equivalent parameters of composition, conditions, and methods of preparing such fusion proteins without departing from the spirit or scope of the invention or any embodiment thereof.

Claims

1. A fusion protein gene comprising 1) the DNA sequence of CD4, or fragment thereof which binds to HIV gp120, and 2) the DNA sequence of an immunoglobulin heavy chain, characterized in that the DNA sequence which encodes the variable region of said immunoglobulin chain has been replaced with the DNA sequence which encodes CD4, or said gp120 binding fragment thereof.

2. The fusion protein gene of claim 1, wherein the DNA sequence which encodes said fragment of CD4 comprises the following DNA sequence:

```
                                           CAATGAACCGGG
                                         -+---------- 120
                                           GTTACTTGGCCC


     GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
121  ---------+---------+---------+---------+---------+---------- 180
     CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


     AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
181  ---------+---------+---------+---------+---------+---------- 240
     TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA


     CCCAGAAGAAGAGCATACAATTCCACTGGAAAAAACTCCAACCAGATAAAGATTCTGGGAA
241  ---------+---------+---------+---------+---------+---------- 300
     GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT


     ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
301  ---------+---------+---------+---------+---------+---------- 360
     TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT


     GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
361  ---------+---------+---------+---------+---------+---------- 420
     CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA


     CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
421  ---------+---------+---------+---------+---------+---------- 480
     GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC


     GATTGACTGCCAACTCTGACACCCACCTGCTTC
481  ---------+---------+---------+--- 
     CTAACTGACGGTTGAGACTGTGGGTGGACGAAG
```

or a degenerate variant thereof, or the following DNA sequence:

```
                                        CAATGAACCGGG
                                        -+---------- 120
                                        GTTACTTGGCCC


        GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
    121 ---------+---------+---------+---------+---------+--------- 180
        CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


        AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
    181 ---------+---------+---------+---------+---------+--------- 240
        TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA


        CCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAA
    241 ---------+---------+---------+---------+---------+--------- 300
        GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT


        ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
    301 ---------+---------+---------+---------+---------+--------- 360
        TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT


        GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
    361 ---------+---------+---------+---------+---------+--------- 420
        CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA


        CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
    421 ---------+---------+---------+---------+---------+--------- 480
        GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC


        GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
    481 ---------+---------+---------+---------+---------+--------- 540
        CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC


        AGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC
    541 ---------+---------+---------+---------+---------+--------- 600
        TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTTGTATG


        AGGGGGGGAAGACCCTCTCCGTGTCTCAG
    601 ---------+---------+---------
        TCCCCCCCTTCTGGGAGAGGCACAGAGTC
```

or a degenerate variant thereof.

3. The fusion protein gene of claim 1 or 2, characterized in that said immunoglobulin chain is of the class IgM, IgG1 or IgG3.

4. A fusion protein gene comprising 1) the DNA sequence of CD4, or fragment thereof which binds to HIV gp120, and 2) the DNA sequence of an immunoglobulin light chain, characterized in that the DNA sequence which encodes the variable region of said immunoglobulin light chain has been replaced with the DNA sequence which encodes CD4, or HIV gp120-binding fragment thereof.

5. A fusion protein gene of claim 4, characterized in that the DNA sequence which encodes said fragment of CD4 comprises the following DNA sequence:

```
                                     CAATGAACCGGG
                                   -+---------- 120
                                     GTTACTTGGCCC


     GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
121  ---------+---------+---------+---------+---------+---------- 180
     CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG




     AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
181  ---------+---------+---------+---------+---------+---------- 240
     TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA


     CCCAGAAGAAGAGCATACAATTCCACTGGAAAAAACTCCAACCAGATAAAGATTCTGGGAA
241  ---------+---------+---------+---------+---------+---------- 300
     GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT


     ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
301  ---------+---------+---------+---------+---------+---------- 360
     TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT


     GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
361  ---------+---------+---------+---------+---------+---------- 420
     CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA


     CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
421  ---------+---------+---------+---------+---------+---------- 480
     GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC


     GATTGACTGCCAACTCTGACACCCACCTGCTTC
481  --------+---------+---------+---
     CTAACTGACGGTTGAGACTGTGGGTGGACGAAG
```

or a degenerate variant thereof, or the following DNA sequence:

```
                                  CAATGAACCGGG
                                  -+---------- 120
                                  GTTACTTGGCCC

      GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
  121 ---------+---------+---------+---------+---------+--------- 180
      CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


      AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
  181 ---------+---------+---------+---------+---------+--------- 240
      TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA



      CCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAA
  241 ---------+---------+---------+---------+---------+--------- 300
      GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT


      ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
  301 ---------+---------+---------+---------+---------+--------- 360
      TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT


      GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
  361 ---------+---------+---------+---------+---------+--------- 420
      CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA


      CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
  421 ---------+---------+---------+---------+---------+--------- 480
      GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC


      GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
  481 ---------+---------+---------+---------+---------+--------- 540
      CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC


      AGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC
  541 ---------+---------+---------+---------+---------+--------- 600
      TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTTGTATG


      AGGGGGGGAAGACCCTCTCCGTGTCTCAG
  601 ---------+---------+--------- 
      TCCCCCCCTTCTGGGAGAGGCACAGAGTC
```

or a degenerate variant thereof.

6. A vector comprising the fusion protein gene of claim 1, preferably having the identifying characteristics of pCD4Hγ1, which has been deposited under Accession No. 67611, or pCD4Mμ, which has been deposited under Accession No. 67608, or of pCD4Pμ, which has been deposited under Accession No. 67609, or of pCD4Eγ1, which has been deposited under Accession No. 67610, all in E. coli at the ATCC under the terms of the Budapest Treaty.

7. A vector comprising the fusion protein gene of claim 4.

8. A host transformed with the vector of claim 6 or 7.

9. The host of claim 8 which expresses an immunoglobulin light chain together with the expression product of said fusion protein gene to give an immunoglobulin-like molecule which binds to gp120 or an immunoglobulin heavy chain together with the expression product of said fusion protein gene to give an immunoglobulin-like molecule which binds to HIV or SIV gp120.

10. The host of claim 9, wherein said immunoglobulin heavy chain is of the immunoglobulin class IgM, IgG1 or IgG3.

11. A method of producing a fusion protein comprising CD4, or fragment thereof which binds to gp120, and immunoglobulin heavy chain, wherein the variable region of the immunoglobulin chain has been substituted with CD4, or fragment thereof which binds to HIV or SIV gp120, characterized by cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with the vector of claim 6, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and recovering the fusion protein so produced.

12. The method of claim 11, wherein said host strain is a myeloma cell line which produces immunoglobulin light chains and said fusion protein comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein an immunoglobulin-like molecule comprising said fusion protein is produced.

13. A method of producing a fusion protein comprising CD4, or fragment thereof which binds to gp120, and an immunoglobulin light chain, wherein the variable region of the immunoglobulin chain has been substituted with CD4, or fragment thereof which binds to HIV or SIV gp120, characterized by cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with the vector of claim 7, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and recovering the fusion protein so produced.

14. The method of claim 13, wherein said host produces immuno-globulin heavy chains of the class IgM, IgG1 and IgG3 together with said fusion protein to give an immunoglobulin-like molecule which binds to HIV-gp120.

15. A fusion protein, which is preferably detectably labeled, comprising CD4, or fragment thereof which is capable of binding to HIV or SIV gp120, fused at the C-terminus to a second protein which comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein the variable region of said heavy chain immunoglobulin has been replaced with CD4, or HIV gp120-binding fragment thereof, and preferably further comprising a therapeutic agent, radiolabel or NMR imaging agent linked to said fusion protein.

16. The fusion proteins CD4Hγ1, CD4Mμ, CD4Pμ, CD4Eγ1 or CD4Bγ1.

17. An immunoglobulin-like molecule, comprising the fusion protein of claim 15 and an immunoglobulin light chain, preferably further comprising a detectable label, and especially further comprising a therapeutic agent, radiolabel or NMR imaging agent linked to said immunoglobulin-like molecule.

18. A fusion protein comprising CD4, or fragment thereof which binds to HIV gp120, fused at the C-terminus to a second protein comprising an immunoglobulin light chain where the variable region has been deleted, and which fusion protein preferably is detectably labeled, especially further comprising a therapeutic agent, radiolabel or NMR imaging agent linked to said fusion protein.

19. The fusion protein of claim 15, wherein said CD4 fragment comprises the following amino acid sequence:

```
                                              M N R G
             V P F R H L L L V L Q L A L L P A A T Q
             G K K V V L G K K G D T V E L T C T A S
 5           Q K K S I Q F H W K N S N Q I K I L G N
             Q G S F L T K G P S K L N D R A D S R R
             S L W D Q G N F P L I I K N L K I E D S
10           D T Y I C E V E D Q K E E V Q L L V F G
             L T A N S D T H L L Q
```

15   or the following amino acid sequence:


```
                                              M N R G
             V P F R H L L L V L Q L A L L P A A T Q
20           G K K V V L G K K G D T V E L T C T A S
             Q K K S I Q F H W K N S N Q I K I L G N
             Q G S F L T K G P S K L N D R A D S R R
25           S L W D Q G N F P L I I K N L K I E D S
             D T Y I C E V E D Q K E E V Q L L V F G
             L T A N S D T H L L Q G Q S L T L T L E
             S P P G S S P S V Q C R S P R G K N I Q
30           G G K T L S V S Q    .
```

20. An immunoglobulin-like molecule comprising the fusion protein of claim 18 and an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, preferably further comprising a detectable label, and especially
35   further comprising a therapeutic agent, radiolabel or NMR imaging agent linked to said immunoglobulin-like molecule.

21. A complex comprising the fusion protein of claim 15 or 18 and HIV or SIV gp120.

22. The complex of claim 21, wherein said gp120 is a part of an HIV or SIV, is expressed on the surface of an HIV or SIV-infected cell or is present in solution.

40   23. A method for the detection of HIV or SIV gp120 in a sample, characterized by

(a) contacting a sample suspected of containing HIV or SIV gp120 with the fusion protein of claim 15 or 18, and

(b) detecting whether a complex is formed, said fusion protein preferably being detectably labeled.

45

Claims for the following Contracting State: GR

1. A vector comprising a fusion protein gene comprising 1) the DNA sequence of CD4, or fragment
50   thereof which binds to HIV gp120, and 2) the DNA sequence of an immunoglobulin heavy chain, characterized in that the DNA sequence which encodes the variable region of said immunoglobulin chain has been replaced with the DNA sequence which encodes CD4, or said gp120 binding fragment thereof.

2. The vector of claim 1, having the identifying characteristics of pCD4Hγ1, which has been deposited in E. coli at the ATCC under the terms of the Budapest Treaty under Accession No. 67611.

55   3. The vector of claim 1, having the identifying characteristics of pCD4Mμ, which has been deposited in E. coli at the ATCC under the terms of this Budapest Treaty under Accession No. 67608.

4. The vector of claim 1, having the identifying characteristics of PCD4Pμ, which has been deposited in E. coli at the ATCC under the Budapest Treaty under Accession No. 67609.

5. The vector of claim 1, having the identifying characteristics of PC4Eγ1, which has been deposited in E. coli at the ATCC under the terms of the Budapest Treaty under Accession No. 67610.

6. A vector comprising a fusion protein gene characterized by 1) the DNA sequence of CD4, or fragment thereof which binds to HIV gp120, and 2) the DNA sequence of an immunoglobulin light chain, wherein the DNA sequence which encodes the variable region of said immunoglobulin light chain has been replaced with the DNA sequence which encodes CD4, or HIV gp120-binding fragment thereof.

7. A host transformed with the vector of claim 1.

8. The host of claim 7 which expresses an immunoglobulin light chain together with the expression product of said fusion protein gene to give an immunoglobulin-like molecule which binds to gp120.

9. A host transformed with the vector of claim 6.

10. The host of claim 6 which expresses an immunoglobulin heavy chain together with the expression product of said fusion protein gene to give an immunoglobulin-like molecule which binds to HIV or SIV gp120.

11. The host of claim 10, characterized in that said immunoglobulin heavy chain is of the immunoglobulin class IgM, IgG1 or IgG3.

12. A method of producing a fusion protein comprising CD4, or fragment thereof which binds to gp120, and an immunoglobulin heavy chain, wherein the variable region of the immunoglobulin chain has been substituted with CD4, or fragment thereof which binds to HIV or SIV gp120, characterized by cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with the vector of claim 1, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and recovering the fusion protein so produced. —

13. The method of claim 12, characterized in that said host strain is a myeloma cell line which produces immunoglobulin light chains and said fusion protein comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein an immunoglobulin-like molecule comprising said fusion protein is produced.

14. A method of producing a fusion protein comprising CD4, or fragment thereof which binds to gp120, and an immunoglobulin light chain, wherein the variable region of the immunoglobulin chain has been substituted with CD4, or fragment thereof which binds to HIV or SIV gp120, characterized by cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with the vector of claim 6, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and recovering the fusion protein so produced.

15. The method of claim 14, characterized in that said host produces immuno-globulin heavy chains of the class IgM, IgG1 and IgG3 together with said fusion protein to give an immunoglobulin-like molecule which binds to HIV-gp120.

16. A method for the detection of HIV or SIV gp120 in a sample, characterized by

(a) contacting a sample suspected of containing HIV or SIV gp120 with a fusion protein comprising CD4, or fragment thereof which binds to HIV gp120, and 2) an immunoglobulin heavy chain, wherein the variable region of said immunoglobulin chain has been replaced with CD4, or said gp120 binding fragment thereof, and

(b) detecting whether a complex is formed.

17. The method of claim 16, characterized in that said fusion protein is detectably labeled.

18. A method for the detection of HIV or SIV gp120 in a sample, characterized by

(a) contacting a sample suspected of containing HIV or SIV gp120 with a fusion protein comprising comprising 1) CD4, or fragment thereof which binds to HIV gp120, and 2) an immunoglobulin light chain, wherein the variable region of said immunoglobulin light chain has been replaced with CD4, or HIV gp120-binding fragment thereof, and

(b) detecting whether a complex has formed.

19. The method of claim 18, characterized in that said fusion protein is detectably labeled.

Claims for the following Contracting State: ES

1. A method of producing a fusion protein comprising CD4, or fragment thereof which binds to gp120, and an immunoglobulin heavy chain, wherein the variable region of the immunoglobulin chain has been substituted with CD4, or fragment thereof which binds to HIV or SIV gp120, characterized by cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with a vector comprising a fusion protein gene comprising 1) the DNA sequence of CD4, or fragment thereof which binds to HIV

gp120, and 2) the DNA sequence of an immunoglobulin heavy chain, wherein the DNA sequence which encodes the variable region of said immunoglobulin chain has been replaced with the DNA sequence which encodes CD4, or said gp120 binding fragment thereof, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and recovering the fusion protein so produced.

2. The method of claim 1, characterized in that said vector has the identifying characteristics of pCD4Hγ1, which has been deposited in E. coli at the ATCC under the terms of the Budapest Treaty under Accession No. 67611.

3. The method of claim 1, characterized in that said vector has the identifying characteristics of PCD4Mμ, which has been deposited in E. coli at the ATCC under the terms of this Budapest Treaty under Accession No. 67608.

4. The method of claim 1, characterized in that said vector has the identifying characteristics of PCD4Pμ, which has been deposited in E. coli at the ATCC under the Budapest Treaty under Accession No. 67609.

5. The method of claim 1, characterized in that said vector has the identifying characteristics of pCD4Eγ1, which has been deposited in E. coli at the ATCC under the terms of the Budapest Treaty under Accession No. 67610.

6. The method of claim 1, characterized in that said host strain is a myeloma cell line which produces immunoglobulin light chains and said fusion protein comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein an immunoglobulin-like molecule comprising said fusion protein is produced.

7. A method of producing a fusion protein comprising CD4, or fragment thereof which binds to gp120, and an immunoglobulin light chain, wherein the variable region of the immunoglobulin chain has been substituted with CD4, or fragment thereof which binds to HIV or SIV gp120, characterized by cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with a vector comprising a fusion protein gene comprising 1) the DNA sequence of CD4, or fragment thereof which binds to HIV gp120, and 2) the DNA sequence of an immunoglobulin light chain, wherein the DNA sequence which encodes the variable region of said immunoglobulin light chain has been replaced with the DNA sequence which encodes CD4, or HIV gp120-binding fragment thereof, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and recovering the fusion protein so produced.

8. The method any one of claims 1 or 7, characterized in that the DNA sequence which encodes said fragment of CD4 comprises the following DNA sequence:

```
                                                    CAATGAACCGGG
                                                    -+---------- 120
5                                                   GTTACTTGGCCC


      GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
121   ---------+---------+---------+---------+---------+--------- 180
10    CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


      AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
181   ---------+---------+---------+---------+---------+--------- 240
15    TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA


      CCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAA
241   ---------+---------+---------+---------+---------+--------- 300
20    GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT


      ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
301   ---------+---------+---------+---------+---------+--------- 360
25    TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT


      GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
361   ---------+---------+---------+---------+---------+--------- 420
30    CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA


      CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
421   ---------+---------+---------+---------+---------+--------- 480
35    GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC


      GATTGACTGCCAACTCTGACACCCACCTGCTTC
481   ---------+---------+---------+---
40    CTAACTGACGGTTGAGACTGTGGGTGGACGAAG


or a degenerate variant thereof.
      9. The method of any one of claims 1 or 7, characterized in that said DNA sequence which encodes
45 said fragment of CD4 comprises the following DNA sequence:
```

```
                              CAATGAACCGGG

                              -+---------- 120

                              GTTACTTGGCCC


     GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC

121  ---------+---------+---------+---------+---------+--------- 180

     CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG


     AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGGATACAGTGGAACTGACCTGTACAGCTT

181  ---------+---------+---------+---------+---------+--------- 240

     TCCCTTTCTTTCACCACGACCCGTTTTTTCCCCTATGTCACCTTGACTGGACATGTCGAA


     CCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAA

241  ---------+---------+---------+---------+---------+--------- 300

     GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT


     ATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA

301  ---------+---------+---------+---------+---------+--------- 360

     TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTCGACTTACTAGCGCGACTGAGTTCTT


     GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT

361  ---------+---------+---------+---------+---------+--------- 420

     CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA


     CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG

421  ---------+---------+---------+---------+---------+--------- 480

     GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCACGTTAACGATCACAAGC


     GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG

481  ---------+---------+---------+---------+---------+--------- 540

     CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC


     AGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC

541  ---------+---------+---------+---------+---------+--------- 600

     TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTTGTATG


     AGGGGGGGAAGACCCTCTCCGTGTCTCAG

601  ---------+---------+---------

     TCCCCCCCTTCTGGGAGAGGCACAGAGTC
```

or a degenerate variant thereof.

10. The method of claim 7, characterized in that said host produces immuno-globulin heavy chains of the class IgM, IgG1 and IgG3 together with said fusion protein to give an immunoglobulin-like molecule which binds to HIV-gp120.